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Protective effects of apigenin, a bioflavonoid, on Benzo[a]Pyrene-Induced lung injury via modulation of oxidative stress and inflammatory responses

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Editorial Office: editor@ffhdj.com

Submission Date: March 18th, 2026; Acceptance Date: April 18th, 2026; Publication Date: April 23th, 2026

Please cite this article as: Alhoshaiyan A. M. A., Alkhalil M. Y. I., Aldakhil A. I., Alharbi H. O. A. Protective effects of apigenin, a bioflavonoid, on Benzo[a]Pyrene-induced lung injury via modulation of oxidative stress and inflammatory responses. *Functional Foods in Health and Disease* 2026; 16(4): 360 – 374
DOI: https://doi.org/10.31989/ffhd.v16i4.1962

ABSTRACT

Background: Apigenin (API) is a flavonoid known for its antioxidant as well as anti-inflammatory effects, which help prevent and slow the progression of pathogenesis.

Objectives: This study aimed to evaluate the lung-protective effect of API in benzo[a]pyrene (BaP)-induced lung injury.

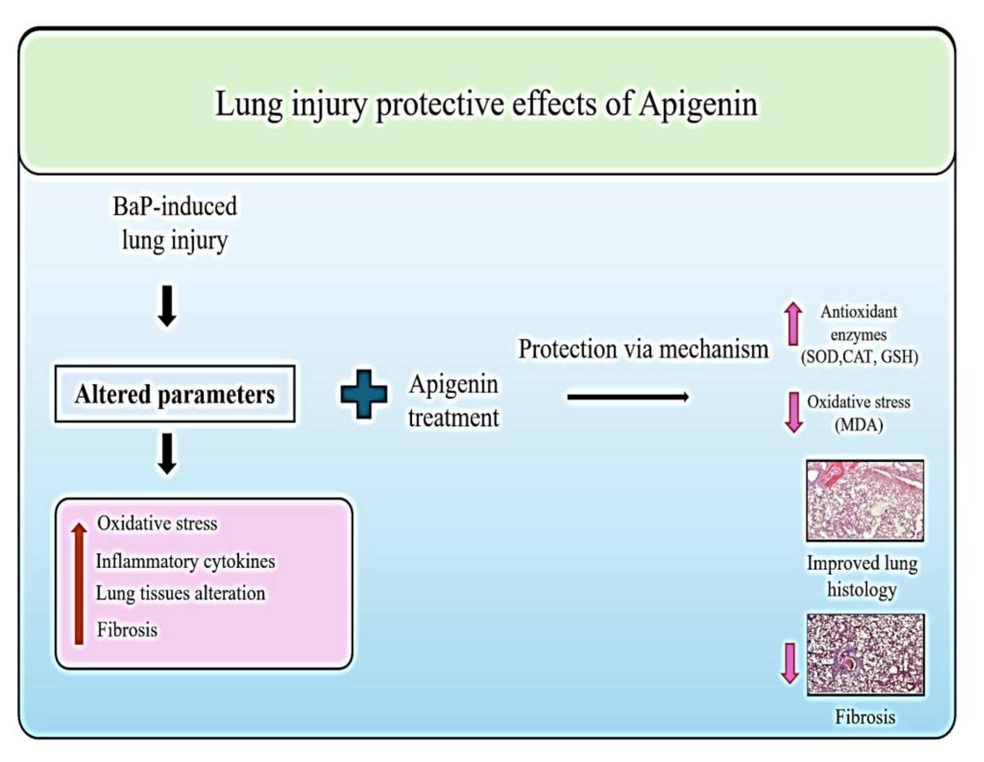
Methods: Biochemical analyses were conducted to evaluate oxidative stress and inflammation in different experimental groups. Furthermore, lung tissue architecture and fibrosis were examined through histopathological analysis. Immunohistochemical staining was also performed to assess interleukin-6 (IL-6) expression.

Results: The BaP-administration group showed decreased antioxidant enzyme levels and increased levels of inflammatory markers. These adverse effects on lung tissue were reversed by API treatment. Moreover, API significantly reduced lung tissue alterations, including congestion, inflammation, hemorrhage, and fibrosis, compared to the BaP-administered group. Moreover, lung tissue from the control group showed no IL-6 protein expression, whereas BaP-treated rats exhibited strong cytoplasmic IL-6 expression. In the BaP + API-treated group, a marked reduction in IL-6 immunoreactivity was observed compared to the BaP-treated group. The findings of this study demonstrate that API possesses antioxidant and anti-inflammatory potential and restores lung tissue architecture.

Conclusion: These results suggest that apigenin could serve as a promising natural therapeutic agent for lung-associated pathogenesis. Therefore, these findings indicate that apigenin may act as an auspicious natural therapeutic agent for lung-related pathogenesis.

Novelty of the Study: This study demonstrates that apigenin (API) protects against BaP-induced lung injury. API administration was associated with reduced oxidative stress and inflammation, as demonstrated by the restoration of antioxidant enzyme activities, reduced pro-inflammatory cytokine expression, and improved lung tissue architecture. These results reveal the potential of API as a natural therapeutic agent and deliver a basis for its future application in functional foods and nutraceuticals targeting lung health.

Keywords: Apigenin; Benzo[a]pyrene; Oxidative stress; Lung injury; Pulmonary fibrosis; Interleukin-6 (IL-6); Functional foods.



Graphical Abstract: Protective effects of apigenin, a bioflavonoid, on Benzo[a]Pyrene-induced lung injury via modulation of oxidative stress and inflammatory responses.

INTRODUCTION

Lung diseases caused by organic pollutants, such as PAHs, are a serious issue [1-2]. These pollutants are metabolized by CYP1A, which can lead to adverse effects such as oxidative stress, inflammation, cancer development, endocrine disruption, and immune system disorders [3-4]. Moreover, these substances can trigger increased CYP1A1 expression, accelerating the metabolism of pollutants in the lungs and leading to even more severe toxic effects [5].

Benzo[a]pyrene (BaP) is one of the most powerful and widely studied PAH is,PAHs, commonly present in the environment [6]. Benzo[a]pyrene (BaP), which accounts which accounts for approximately 22.5–69.8% of tobacco-derived metabolites, has been shown to promote cell proliferation, inflammation, apoptosis, as well as DNA damage, all of which contribute to the development of lung cancer [7]. The toxic effects of (BaP) are mainly mediated by the generation of reactive oxygen species (ROS) and activation of inflammatory signaling pathways [8-9]. Additionally, (BaP) disrupts pulmonary surfactant function, alters lipid metabolism, impairs endothelium-dependent vasodilation and affects various cellular signaling pathways and microRNAs, as demonstrated in both in vivo and in vitro models [10–12]. Numerous studies have shown that the significant adverse health effects associated with (BaP) are connected to increased oxidative stress as well as inflammation [13-16].

Although substantial progress has been made in developing pharmacological interventions for lung diseases, the related mortality rate remains high. Preventing lung pathogenesis as well as reducing disease-related mortality may be achieved through medicinal plants and their bioactive compounds, which are promising therapeutic alternatives due to their minimal adverse side effects.

Epidemiological studies suggest that consuming a diet rich in polyphenols can significantly improve quality of life and may enhance survival in individuals affected by various chronic diseases [17–18]. Moreover, a recent study has confirmed the role of the phytomarine R-L compound in protecting against pulmonary injury in mice through its anti-inflammatory potential [19].

Apigenin occurs naturally in several plant sources, including parsley, celery, and oregano, with the highest concentrations typically present in their dried forms [20]. Apigenin (API) exerts a protective effect against various diseases by mitigating oxidative stress, suppressing inflammatory responses, and regulating multiple cellular and molecular pathways involved in disease progression [21]. The antioxidant and anti-inflammatory properties of apigenin were measured in a mouse model of acute liver injury induced by carbon tetrachloride (CCl_4). In vivo, apigenin significantly reduced AST and ALT levels in CCl_4-treated mice and clearly reduced lipid peroxidation, as evidenced by elevated antioxidant enzyme activities and reduced malondialdehyde levels in liver tissues. Also, apigenin effectively alleviated inflammation. Consistently, apigenin restored elevated AST and ALT levels, the imbalance between GSH and SOD activity, and excessive ROS [22]. The effect of apigenin (API) on arthritis was assessed. Results demonstrated that API distinctly downregulated P2X7/NF-κB signaling–related proteins and attenuated inflammatory responses [23]. The current study was designed to investigate the potential protective effects of B[a]P-induced lung injury in rats by evaluating oxidative stress markers, inflammatory mediators, as well as histopathological changes in lung tissues.

MATERIALS AND METHODS

The general chemicals were of analytical grade and sourced from a local supplier in Saudi Arabia. Benzopyrene and apigenin were bought from Sigma-

Aldrich. Commercial assay kits for assessing antioxidant enzyme activity and inflammatory biomarkers were bought from Abcam, United Kingdom. Antibodies targeting IL-6, in addition to an HRP/DAB immunohistochemistry detection kit, were likewise obtained from the same supplier.

Animals Grouping and Treatment Plan: Rats weighing 160–180 g were found from were obtained from KSU, Kingdom of Saudi Arabia. Upon arrival, the animals were kept for a one-week acclimatization period to minimize transportation-related stress. They were housed at eight rats per cage under standard laboratory conditions, maintained on a regular chow diet. Following acclimatization, the animals were randomly allocated to experimental groups using a randomization method to reduce selection bias. The experimental protocol was conducted over a period of six consecutive weeks. Ethical approval from Qassim University was obtained with number QU-J-UG -2-2025-54308.

Rats were randomly divided into four groups (n = 8 animals per group).

Group I (Normal control): Rats received only the vehicle.

Group II (Disease control): Rats were administered BaP at a dose of 50 mg/kg [24] b.w. orally, twice weekly, for six consecutive weeks.

Group III (API + BaP treatment): Rats in this group received apigenin (API) orally at 20 mg/kg body weight [25] before being treated with BaP at 50 mg/kg, administered twice weekly over a six-week period.

Group IV (API treatment group): Rats received apigenin (20 mg/kg b.w) orally alone.

Sample Collection and Processing: At the end of the treatment, all rats were anesthetized with ether and then sacrificed. Blood as well as lung tissue samples were collected for biochemical, histopathological, and protein expression studies to evaluate the effects of apigenin on lung tissue. Blood samples were centrifuged at 4000 rpm for 10 minutes to obtain serum, which was then stored at −20 °C for subsequent biochemical analysis. A portion of the lung tissue was homogenized in phosphate-buffered saline, and the homogenate was centrifuged at 3500 rpm for 10 minutes to obtain the supernatant, which was stored at −20 °C for further biochemical analysis. The remaining lung tissue samples were fixed in 10% formalin for 2 days to confirm proper preservation, followed by processing in an automated tissue processor to produce paraffin blocks for histopathological investigation and protein expression analysis.

Determination of Inflammatory Marker: To measure the presence of pro-inflammatory cytokines, ELISA test was conducted according to the manufacturer's guidelines. This research involved a comprehensive examination of lung tissue samples from four different experimental groups to quantify the concentrations of Interleukin-1 beta (IL-1β), interleukin-6 (IL-6), as well as tumor necrosis factor-alpha (TNF-α). The data collected from these analyses were interpreted to measure the efficiency of potential therapeutic interventions designed to alleviate these inflammatory responses.

Determination of Antioxidant Enzymes in Different Experimental Groups: Lung homogenates were prepared in a buffer saline to confirm the integrity of the samples. Centrifugation was performed; the supernatant was collected for further analysis. Subsequently, the activities and levels of antioxidant markers were quantitatively measured. The markers such as glutathione, (GSH), superoxide dismutase (SOD), and catalase (CAT), were measured as instructed by the manufacturer. Data derived from these measurements were analyzed.

Evaluation of Lung Tissue Architecture using Hematoxylin and Eosin (H&E) Staining: Formalin 10% was used to fix the lung tissues for 2 days to ensure proper fixation. After fixation, the tissues were processed using tissue processor and embedded in paraffin wax. Sections 5 µm thick were cut from the paraffin-embedded block, and hematoxylin and eosin (H&E) staining was applied to the tissue. A total of four to five randomly selected fields were chosen and analyzed. The stained sections were then photographed with a microscope fitted with a digital camera. Tissue alterations, including hemorrhage, inflammation, and congestion, were analyzed to evaluate the lung-protective effect of apigenin. All histological assessments were performed in a blinded manner by an experienced pathologist. For each sample, 4-5 fields per section were examined under a light microscope to evaluate the pathological changes.

The Fibrosis Evaluation using Masson's Trichrome as well as Sirius red staining: In this study, fibrosis levels across different experimental groups were evaluated according to the company's instructions (Abcam, UK), with slight modifications to enhance staining quality. Collagen accumulation was quantified based on Masson's trichrome-stained tissue sections, where it was indicated by blue staining and was considered as fibrosis. Moreover, collagen fibers were evaluated by Sirius Red staining, which showed red staining. The stained tissue sections were examined as well as photographed. The results were analyzed to evaluate the antifibrotic effect of apigenin.

Immunohistochemistry (IHC) staining for evaluation of protein expression: To assess inflammatory activity, IL-6 protein expression was assessed as previously described [26]. Briefly, lung tissue sections were deparaffinized by xylene and rehydrated through a graded alcohol series. Endogenous peroxidase activity was blocked by 3% hydrogen peroxide. Antigen retrieval was then done by autoclaving the sections in citrate buffer (pH 6.0). Non-specific binding sites were blocked with a protein-blocking solution, followed by washing with PBS. Sections were then incubated overnight at 4°C in a humidified chamber with the IL-6 primary antibody. After three PBS washes, a biotin-conjugated secondary antibody was added, followed by incubation with streptavidin–peroxidase for 15 minutes. Immunoreactivity was visualized by diaminobenzidine (DAB) as the chromogenic substrate, followed by counterstaining by hematoxylin. Immunohistochemical expression of interleukin-6 (IL-6) was evaluated quantitatively. For each tissue section, 4–5 fields were selected and examined under a light microscope. A total of 500 cells per section were counted, and the proportion of positively stained cells was determined. The intensity of IL-6 immunostaining was categorized as mild, moderate, or strong based on staining intensity. The percentage of positively stained cells was calculated, and the mean percentage per sample was determined. All evaluations were performed in a blind manner to avoid observer bias.

Statistical Analysis: The outcomes are presented as mean ± standard deviation (SD). Statistical differences among the investigational groups were assessed using one-way analysis of variance (ANOVA). All analyses were performed using SPSS software, and a p-value of less than 0.05.

RESULTS

Lung protective effects of apigenin were evidenced through different biochemicals and histopathological experimentations. The results are presented as follows:

Impact of apigenin on oxidative stress and the levels of antioxidant enzymes: It was observed that Bap administration led to a significantly higher level of malondialdehyde (MDA). Conversely, co-administration of Bap with API significantly reduced MDA levels as compared to rats treated with Bap alone [Figure 1].

Bap administration caused a noteworthy reduction in antioxidant enzyme levels, whereas co-treatment with Bap as well as API evidently elevated enzyme levels as compared to rats receiving only Bap [Figure 2].

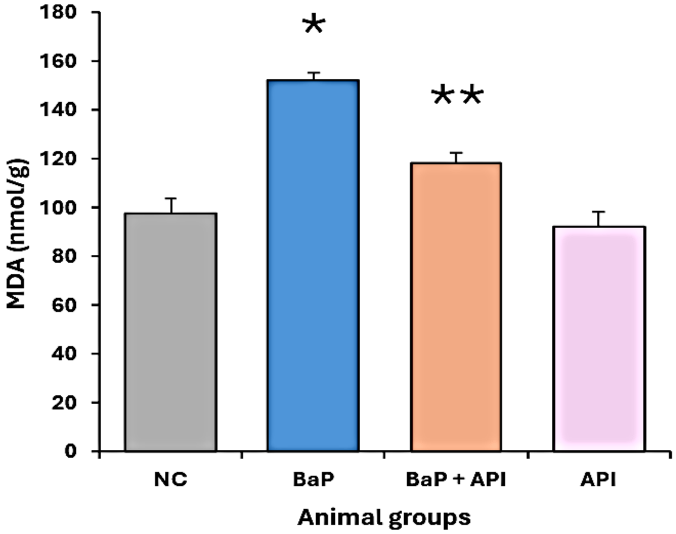


Figure 1. Levels of MDA in control, Bap-administered, Bap + API-treated, and API-only treated rats determined. The number signifies the mean ± SEM. Statistical differences are specified by asterisks: * denotes a significant difference compared with the control group ($p < 0.05$), whereas ** shows a significant difference compared with the BaP-treated group.

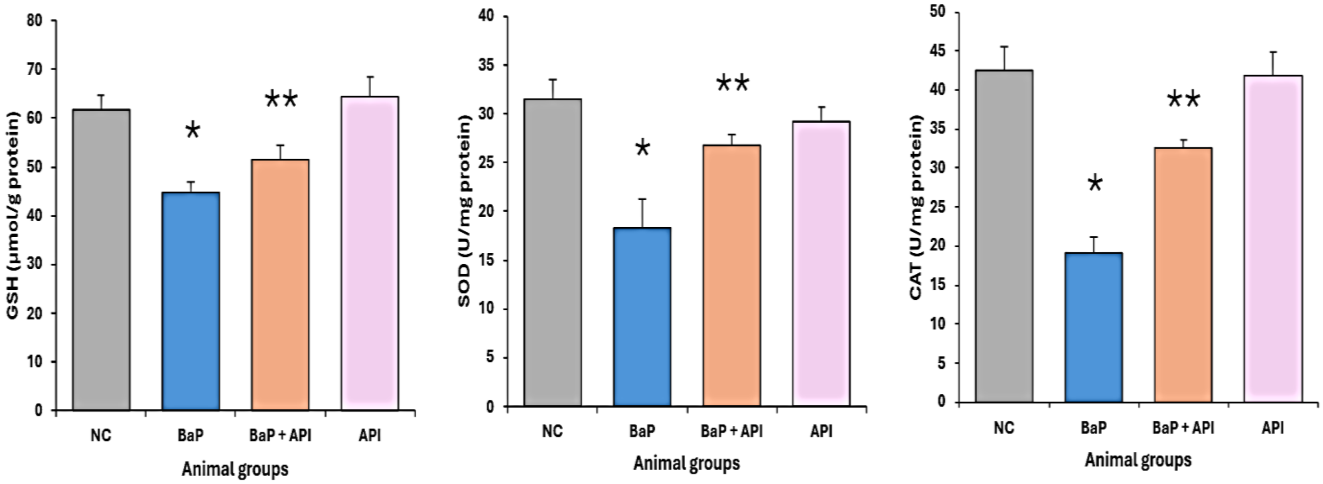


Figure 2. Levels of antioxidant enzymes in control, Bap-administered, Bap + API-treated, and API-only treated rats determined. Statistical differences are specified by * displaying significance when compared to the control group ($p < 0.05$), while ** implies a significant difference from the Bap-administered group.

Effect of Apigenin on inflammatory marker levels: The administration of Bap led to a significantly elevated level of inflammatory markers. In contrast, combining Bap with API treatment led to a considerable decrease in these inflammatory markers compared with Bap-treated rats [Figure 3]. This suggests that apigenin may have a protective effect against inflammation caused by Bap.

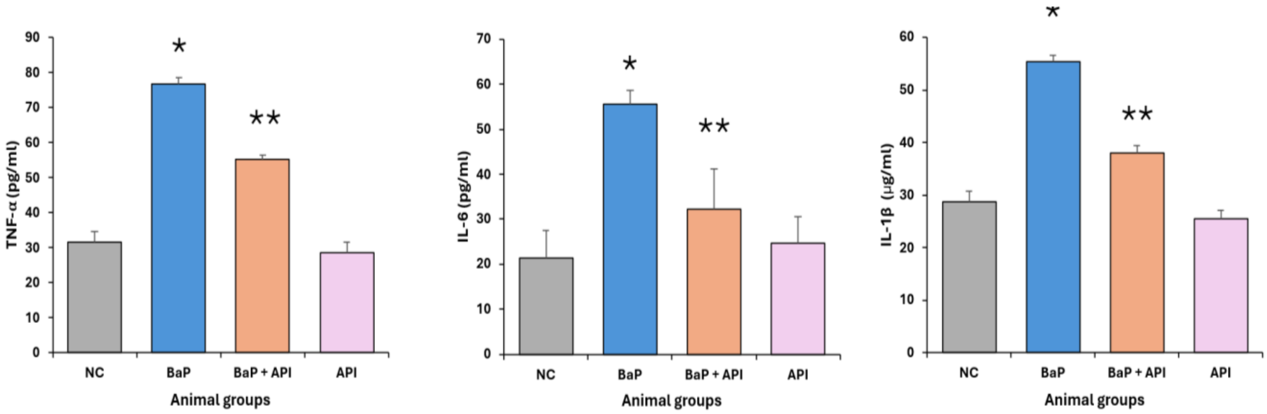


Figure 3. The levels of inflammatory markers (were determined in control, Bap-treated, Bap + API-treated, and API-only treated rats. Statistical significance is specified by asterisks: * signifies a significant difference compared to the control group ($p < 0.05$), whereas ** indicates a significant difference relative to the Bap-administered group.

Effect of Apigenin on lung tissue architecture : The apigenin effect on lung tissue was assessed to measure histopathological changes across the experimental groups. Histological investigation of lung tissue in the control group revealed normal architecture, characterized by well-preserved alveolar spaces, intact bronchial epithelium, and intact interalveolar septa. While rats treated with BaP exhibited noticeable histopathological alterations, including altered lung tissue architecture, congestion, infiltration of inflammatory cells as well as hemorrhages. In the BaP + API-treated group, a noteworthy decrease in inflammatory cell infiltration, congestion, and hemorrhage was observed, along with restoration of normal lung architecture. The results of this study suggest that apigenin has a protective effect against lung damage caused by BaP, evidenced by the preservation of the lung tissue architecture [Figure 4]

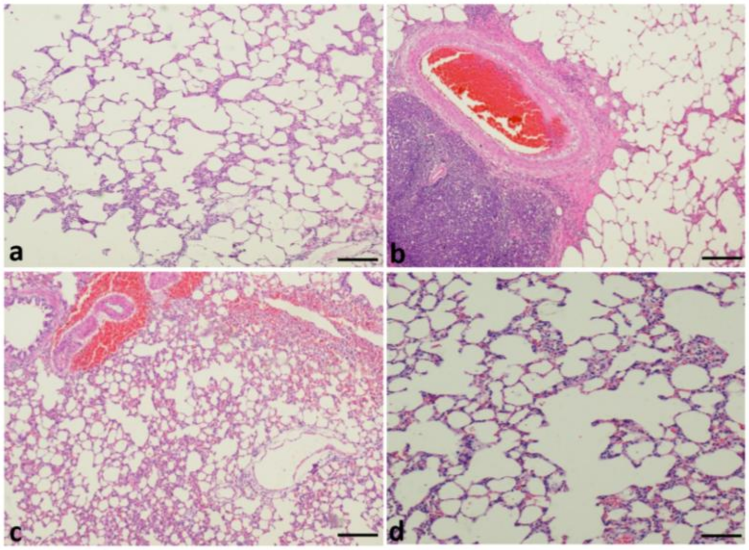


Figure 4. Histopathological examination of lung tissues in (a) The normal lung histological with well-preserved alveolar spaces, and normal interalveolar septa was detected in control group. (b) BaP group: Rats administered benzo[a]pyrene (BaP) exhibited marked histopathological alterations, including disrupted lung architecture, alveolar congestion, hemorrhages, and prominent infiltration of inflammatory cells. (c) BaP + API group: Co-treatment with API markedly attenuated BaP-induced lung injury, as evidenced by reduced inflammatory cell infiltration, decreased congestion, and substantial restoration of normal alveolar architecture. (d) API group: Lung tissues from rats treated with apigenin alone displayed normal histological features, indicating no observable adverse effects. Original magnification: 100×; scale bar: 50 µm.

The impact of Apigenin on lung tissue fibrosis: The impact of apigenin on lung fibrosis was determined by using Masson's trichrome as well as Sirius Red staining to evaluate collagen deposition across the experimental groups. Lung tissue in the control group exhibited normal histological features with minimal collagen deposition, or fibrosis, while rats treated with BaP showed significant fibrotic deposition characterized by bundles of collagen fibers. These appeared as intense blue staining by Masson's trichrome [Figure 5].

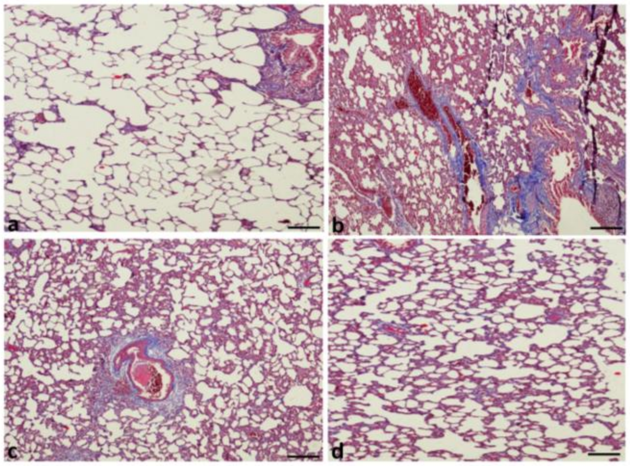


Figure 5. Evaluation of fibrosis by Masson trichrome staining. (a) Control group: Lung sections showing no fibrosis. (b) BaP group: Rats administered exhibited deposition of collagen fiber. (c) BaP + API group: Co-treatment with API markedly attenuated fibrosis, as evidenced by reduced fibrosis. (d) API group: Lung tissues from rats treated with apigenin alone displayed no fibrosis. Original magnification: 100×; scale bar: 50 µm.

The fiber deposition was absent in the control group. while rats administered by BaP showed noteworthy fiber deposition. These appeared as red color staining by Sirius red staining. In the BaP + API treated group, an obvious reduction in fibrosis was seen. The fibrotic areas were noticeably reduced compared to the BaP-treated group, suggesting attenuation of fibrosis [Figure 6]. These results indicate that apigenin provides a protective effect against lung damage caused by BaP through reducing the accumulation of collagen and preventing fibrosis.

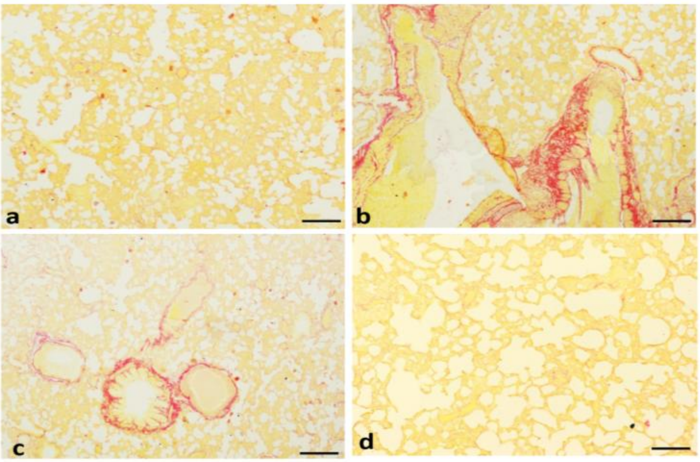


Figure 6. Evaluation of fibrosis by Sirius red staining. (a) Control group: Lung sections showing no fibrosis. (b) BaP group: Rats administered BaP exhibited showing high fibrosis. (c) BaP + API group: Co-treatment with API markedly attenuated fibrosis, as demonstrated by reduced fibrosis. (d) API group: Lung tissues from rats treated with API alone exhibited no fibrosis. Original magnification: 100×; scale bar: 50 µm.

Effect of Apigenin on inflammatory protein expression: T The impact of API on expression of inflammatory protein was evaluated by immunohistochemical (IHC) staining [Figure 7]. Control group lung tissue exhibited no expression of interleukin-6 (IL-6) protein, whereas rats administered BaP exhibited cytoplasmic expression of IL-6 protein, as evidenced by intense brown immunostaining in lung tissue sections. In the BaP + API treated group, noticeable reduction in IL-6 immunoreactivity was detected. The decreased intensity of brown staining suggests attenuation of inflammatory protein expression.

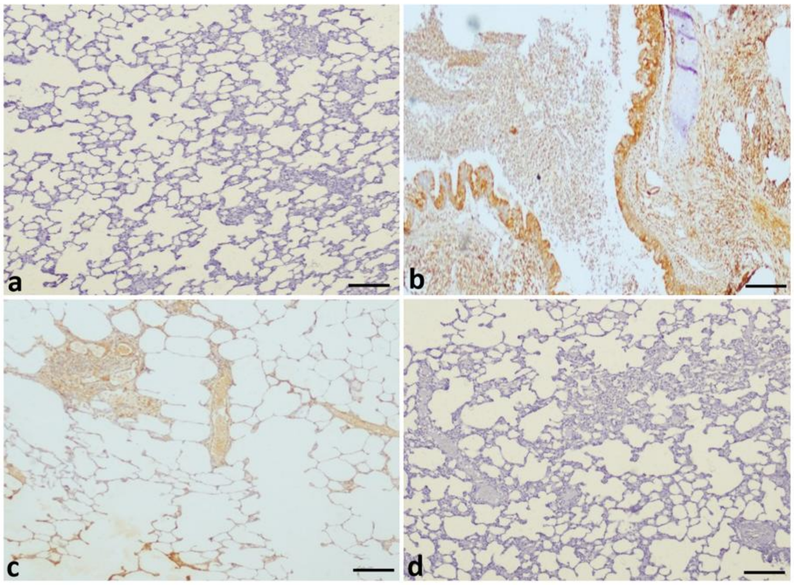


Figure 7. Evaluation of expression of IL-6 protein by immunohistochemistry staining. (a) Control group: Lung sections showing no expression of IL-6 protein. (b) BaP group: Rats administered BaP exhibited high expression. (c) BaP + API group: Co-treatment with API markedly decreased expression of protein. (d) API group: Lung tissues from rats treated with API alone displayed no expression. Original magnification: 100×; scale bar: 50 µm.

DISCUSSION

Benzo(a)pyrene is a polycyclic aromatic hydrocarbon (PAH) generally produced during the incomplete combustion of organic materials and industrial emissions. Exposure to this compound has been associated with increased oxidative stress, DNA damage, and inflammation, which may contribute to the development of diseases. Several studies have shown that increased exposure to air pollutants (BaP) is associated with detrimental health outcomes, leading to tissue damage and increased rates of morbidity and mortality [27-29]. However, the precise mechanisms underlying this association remain inadequately understood.

Lung injury is linked with significant alterations in multiple cellular signaling and metabolic pathways. These changes often involve the activation of inflammatory cascades, oxidative stress responses, and dysregulation of immune signaling, which collectively contribute to tissue damage and impaired pulmonary function. Previous investigations have confirmed that lung injury is associated with alterations in various cell-signaling and metabolic pathways [27,29]. Bap administration leads to inflammation, oxidative stress, as well as changes in lung tissue. Findings indicate that inflammation, driven by the expression of inflammatory cytokines like TNF-α and IL-6, plays a significant role in the development of lung injury [30-31]. The study has recognized a close relationship between oxidative stress as well as inflammatory responses [32]. The pro-inflammatory cytokine, which then initiates and amplifies a cascade of inflammatory reactions [32-33].

In this study, BaP administration was found to decrease the activity of antioxidant enzymes whereas simultaneously increasing the levels of inflammatory cytokines. Conversely, administering Bap alongside apigenin treatment resulted in a marked increase in antioxidant enzyme levels and a decrease in inflammatory cytokine levels. In this regard, previous findings reported that administration of Bap caused increase in inflammatory markers and decrease in antioxidant enzymes [24]. Moreover, the outcomes of this study are in line with previous reports demonstrating that activities of antioxidant enzyme decrease in lung tissue following B(a)P treatment [34]. A previous study confirmed that bleomycin exposure raised myeloperoxidase (MPO) activity and increased the levels of pro-inflammatory cytokines, while simultaneously reducing SOD activity in rat lung tissues. However, treatment with apigenin significantly mitigated these effects by lowering MPO activity and the concentrations of TGF-β and TNF-α, as well as restoring SOD activity that was suppressed by bleomycin administration [25].

The current findings demonstrate that a histological examination of lung tissue of control group confirmed a normal tissue architecture, while rats treated with BaP showed significant histopathological changes. In the group treated with both BaP and apigenin, there was a noticeable reduction in these histological changes, suggesting a restoration towards normal lung architecture. Besides, rats treated with BaP showed significant fibrotic deposition, whereas treatment with apigenin led to a decrease in fibrosis. These results suggest that apigenin provides a protective effect against BaP-induced lung injury by mitigating collagen accumulation and fibrosis. Earlier studies have established that exposure to BaP leads to noteworthy damage to the alveolar structure [11]. Other noted alterations in BaP-treated groups include congestion, fibrosis, and hemorrhage [35]. Similarly, research has demonstrated that BaP-treated groups showed more damage to the alveolar structure, with protective effects observed from compounds such as diosmin and thymoquinone [36,37]. The role of API in treating LIRI-induced pulmonary fibrosis was investigated. It was observed that APG pretreatment relieves pulmonary pathological damage and functional abnormalities in rats [38]. In addition, administration of APG improves LIRI-induced fibrosis levels. API inhibited the formation of pulmonary fibrosis caused by bleomycin in a mouse model [39].

Another study examined the role of apigenin against paraquat (PQ)-induced lung injury. The findings exhibited that PQ-treated mice developed pronounced inflammatory changes in lung tissues, characterized by hemorrhage, edema, inflammatory cell infiltration, narrowing of alveolar spaces, and damage to alveolar structures. However, treatment with apigenin noticeably alleviated these histopathological changes and improved the lung tissue architecture affected by exposure to PQ [40]. Moreover, anti-inflammatory potential of API in acute lung injury has been demonstrated [41].

Oxidative stress plays a critical role in initiating and amplifying inflammatory responses within the body. Elevated ROS levels can damage essential cellular components, leading to cellular dysfunction and tissue injury. The persistent oxidative stress-induced inflammation contributes to the development of disease including lung pathogenesis. Oxidative stress induces inflammation, which subsequently activates NF-Kb [39, 42]. There are many reports that have evidenced health-beneficial role of natural products and bioactive compounds [43-44]. Moreover, recent study discussed the role of plant polyphenols in medicine [45] and protective effects of Ginger or Omega-3 reported [46].

In this study, lung tissues from the BaP-treated group exhibited high IL-6 protein expression. However, rats receiving both BaP and apigenin revealed a noticeable decrease in IL-6 immunoreactivity. The reduced brown staining intensity indicates that apigenin suppressed the expression of inflammatory proteins. Another study reported that exposure to B[a]P significantly elevated the levels of pro-inflammatory markers, in mice compared with vehicle-treated control animals [37]. In this context, it has been shown that certain natural compounds, mainly flavonoids, possess anti-inflammatory properties and can effectively reduce the expression of these inflammatory proteins [37].

A previous study on acute lung injury confirmed that apigenin suppressed LPS-induced expression of iNOS, COX-2, and various pro-inflammatory cytokines [47]. Additionally, the anti-inflammatory effects of apigenin in lung injury were confirmed in another study, where pretreatment with apigenin prior to intratracheal LPS administration significantly reduced the percentage of neutrophils in bronchoalveolar lavage fluid (BALF) and decreased levels of IL-6, IL-1β, and TNF-α in BALF. These findings suggest that the protective effects of apigenin against LPS-induced acute lung injury may be mediated primarily by the inhibition of COX-2 as well as NF-κB gene expression in lung tissues [48]. This study aligns with the functional food development framework by providing preclinical evidence supporting the bioactivity of apigenin in a lung injury model. The present findings contribute to the early stages of development, particularly mechanism validation and preclinical efficacy assessment. The observed antioxidant, anti-inflammatory, and anti-fibrotic effects support further exploration of apigenin for dosage optimization and safety evaluation in future studies. These results therefore provide a scientific basis for advancing apigenin toward potential functional food or nutraceutical applications.

CONCLUSION

This study reveals that apigenin (API) exerts protective effects against benzo[a]pyrene (BaP)-induced lung injury. API administration was associated with reduced oxidative stress as well as inflammation, as evidenced by the restoration of antioxidant enzyme activities, decreased pro-inflammatory cytokine expression, and improved lung tissue architecture. In addition, API attenuated fibrosis, and histopathological alterations, indicating its combined antioxidant and anti-inflammatory actions. Notably, this study provides further insight into the protective role of apigenin in a BaP-induced lung injury model by integrating biochemical, histopathological, and immunohistochemical findings. The observed reduction in IL-6 expression and improvement in tissue structure suggest that apigenin may modulate inflammatory and oxidative stress–related processes while preserving lung integrity. These findings provide a more comprehensive understanding of the protective role of apigenin, particularly its dual action in attenuating oxidative stress and inflammation alongside preserving lung architecture. In conclusion, apigenin shows promise as a natural compound with protective effects in experimental lung injury, warranting further investigation for its potential application in lung-related disorders.

Abbreviations: API: Apigenin; BALFs: bronchoalveolar lavage fluids; BaP: benzo[a]pyrene; CAT: catalase; DAB:

diaminobenzidine; GST: glutathione S-transferase; IHC: immunohistochemistry; IL-1β: interleukin-1 beta; IL-6: interleukin-6; MDA: malondialdehyde; NF-κB: nuclear factor kappa B; PAH: polycyclic aromatic hydrocarbon; SOD: superoxide dismutase; TNF-α: tumor necrosis factor-alpha.

Competing Interests: The authors declare that they have no conflicts of interest.

Author's Contributions: Conceptualization: A.M.A.A (Abdulaziz Mohammed Ali Alhoshaiyan), M.Y.I.A. (Mohhamed Yousef Ibrahim Alkhalil1), A.I.A (Adel Ibrahim Aldakhil1), H.O.A.A (Hajed Obaid A Alharbi), methodology: A.M.A.A (Abdulaziz Mohammed Ali Alhoshaiyan), M.Y.I.A. (Mohhamed Yousef Ibrahim Alkhalil), A.I.A (Adel Ibrahim Aldakhil), writing-original draft preparation: A.M.A.A (Abdulaziz Mohammed Ali Alhoshaiyan), M.Y.I.A. (Mohhamed Yousef Ibrahim Alkhalil), A.I.A (Adel Ibrahim Aldakhil). writing—review and editing: H.O.A.A (Hajed Obaid A Alharbi). Supervision: H.O.A.A (Hajed Obaid A Alharbi). funding acquisition: H.O.A.A (Hajed Obaid A Alharbi). All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Deanship of Graduate Studies and Scientific Research, Qassim University, Saudi Arabia, under the project number (QU-J-UG-2-2025-54308) during the academic year 1446 AH/2024 AD.

Acknowledgments

The authors gratefully acknowledge Qassim University, represented by the Deanship of Graduate Studies and Scientific Research, on the financial support for this research under the number (QU-J-UG-2-2025-54308) during the academic year 1446 AH/2024 AD.

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