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Protective effect of Shilajit against Bisphenol A-induced pulmonary histopathological alterations in male mice

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Bisphenol A (BPA) is a pervasive industrial environmental pollutant widely used in chemical manufacturing, leading to continuous human and animal exposure through food and resource contamination. While its broad multi-organ toxicities are well-documented, its sub-acute destructive impact on mammalian respiratory histology requires comprehensive elucidation. Conversely, Shilajit is a traditional mineral pitch known for its potent organic constituents. This study evaluated the lung tissue alterations induced by sub-acute oral BPA exposure and investigated the potential protective efficacy of concurrent Shilajit administration in a male mouse model. Thirty healthy adult male mice were randomly assigned to three equal cohorts: vehicle control (G1), toxic exposure group receiving BPA at 400 mg/kg body weight (G2), and co-treatment group receiving BPA concurrently with Shilajit at 0.5 g/kg body weight (G3) via oral gavage for 21 consecutive days. Following euthanasia, longitudinal lung tissue samples were promptly fixed in 10 % neutral buffered formalin for 48 hours, embedded in paraffin blocks, sectioned, and stained with hematoxylin and eosin (H&E). Stained specimens were subjected to qualitative screening and quantitative scoring. Data were statistically evaluated using Kruskal-Wallis, Dunn's post hoc, and Fisher's exact tests. Microscopic evaluation revealed extensive, severe structural damage in 90 % of the BPA-alone group (G2), characterized by diffuse interalveolar septal thickening driven by heavy mononuclear cell (MNC) infiltration, dense accumulation of amorphous proteinaceous exudates within the respiratory spaces, perivascular leukocytic aggregation, and focal alveolar wall rupture driving emphysematous alterations. Conversely, concurrent Shilajit administration (G3) significantly mitigated these changes ($P<0.05$), successfully restoring normal lung histoarchitecture in 70 % of the cohort. The protected parenchyma demonstrated well-preserved alveolar matrices with mild congestion and localized, contained MNC infiltration. Notably, the G3 group uniquely exhibited distinct interstitial granulomatous accumulations and spherical micro-cavities within the microvascular beds, indicating active tissue regeneration.

Keywords: Bisphenol A, Shilajit, pulmonary toxicity, histopathological changes, mice.

Протективний ефект муміє проти індукованих бісфенолом А патоморфологічних змін у легенях самців мишей

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Бісфенол А є поширеною промисловою хімічною речовиною, яка широко використовується у виробництві епоксидних смол і полікарбонатних пластиків, що призводить до постійного впливу на організм людини та тварин через забруднення харчових продуктів і навколишнього середовища. Хоча системна токсичність бісфенолу щодо різних органів добре вивчена, вибірковий підгострий деструктивний вплив на респіраторну систему потребує подальшого вивчення. Відомо, що муміє є традиційним мінеральним фітокомплексом, відомим в аюрведичній медицині завдяки своїм потужним антиоксидантним, протизапальним і протективним властивостям. Метою цього дослідження було оцінити гістопатологічні зміни в легенях, спричинені підгострим пероральним впливом бісфенолу А, та дослідити потенційну терапевтичну й органопротективну ефективність супутнього введення муміє на моделі самців мишей. Тридцять здорових дорослих самців мишей було випадково розподілено на три рівні групи: контрольна група (G1), група, яка отримувала бісфенол А у дозі 400 мг/кг маси тіла (G2), та група сумісного введення бісфенолу А та муміє у дозі 0,5 г/кг маси тіла (G3) шляхом перорального зондування протягом 21 дня. Після евтаназії поздовжні зрізи легень фіксували в 10 % нейтральному забуференому формаліні, фарбували гематоксиліном та еозином і піддавали якісному скринінгу та кількісному гістопатологічному оцінюванню. Мікроскопічна оцінка виявила значні, тяжкі структурні пошкодження у 90 % тварин групи BPA (G2), що характеризувалися дифузним потовщенням міжальвеолярних перегородок внаслідок масивної інфільтрації мононуклеарними клітинами, щільним накопиченням аморфного білкового ексудату в просвітах альвеолярних пухирців, периваскулярною лейкоцитарною агрегацією та вогнищевим розривом альвеолярних стінок із розвитком емфізематозних змін. Навпаки, супутнє введення муміє (G3) суттєво нівелювало ці деструктивні зміни ($P<0,05$), успішно відновлюючи нормальну гістоструктуру легень у 70 % тварин у групі. У тварин, що отримали муміє, паренхіма демонструвала збережену впорядковану будову тонкостінної альвеолярної архітектоніки, виявляючи лише помірне застійне повнокров'я судин та локалізовану, чітко обмежену периваскулярну інфільтрацію мононуклеарними клітинами. Зокрема, у групі G3 виявлено чітко окреслені інтерстиціальні гранулематозні скупчення у поєднанні з ізольованими сферичними мікрокавернами всередині мікроциркуляторного русла, які структурно нагадували початкову стадію жирової мікроемболії, що свідчить про запуск активного, організованого процесу структурної перебудови тканин.

Ключові слова: бісфенол А, муміє, легенева токсичність, гістопатологічні зміни, миші.

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Introduction

Bisphenol A (BPA) is a well-known industrial chemical widely used in the manufacturing of epoxy resins and polycarbonate plastics, which is commonly associated with everyday human exposure via food contamination [1, 2]. Regarding its health hazards, BPA induces chronic inflammation, oxidative stress, DNA damage, and fibrotic reactions in multiple organs [3–5]. Several studies [6, 7] have demonstrated the toxic effects of BPA on the respiratory system, highlighting inflammation, oxidative stress, and apoptosis as the primary pathways involved in BPA-induced lung injury.

The phenomenon of bisphenol leaching from polycarbonate flasks was first discovered in 1993, notably during autoclaving procedures that subsequently triggered the proliferation of breast cancer cells in laboratory settings [8]. Both laboratory animals and humans readily absorb BPA. When ingested orally, its bioavailability and metabolic pathways present complex dynamics: while a portion remains unaltered, more than 90 % of the circulating chemical binds to plasma proteins [9]. As it passes through the liver, the absorbed bisphenol undergoes extensive hepatic metabolism before systemic distribution [10].

Shilajit (commonly known as Mumie, or Hajar ulmusa in Arabic) is a natural mineral pitch that slowly emerges from sedimentary rock layers in high-altitude mountain ranges during warm seasons. It is produced via the protracted humification of specific plant matter over centuries. Phytochemical analyses indicate that 60–70 % of Shilajit consists of a rich organic humus, heavily packed with its principal bioactive constituents: fulvic acid, humic acid, core minerals, and vital trace elements such as selenium, zinc, and iron [11].

While widely celebrated in Ayurvedic traditional medicine for its multi-targeted therapeutic efficacy, the World Health Organization (WHO) has strongly encouraged establishing a rigorous, evidence-based understanding of the safety, quality, and specific organ-protective mechanisms of traditional medicines within modern scientific frameworks [12]. Shilajit's capacity to neutralize dangerous free radicals, block pro-inflammatory cytokine pathways, and promote tissue regeneration marks it as a promising candidate for mitigating chemical-induced toxicity [13].

The aim of the study

Therefore, the purpose of this study was to assess the protective effect of co-administered Shilajit and to investigate the systemic histopathological consequences of sub-acute oral BPA exposure on mouse lung tissue.

Materials and methods

The study was conducted at the Faculty of Veterinary Medicine, University of Kerbala, beginning from 3 January to 24 January, 2026. Specialized cages were utilized for the mice during the experiment.

Animal housing and experimental design

Thirty healthy adult male mice were kept in specialized, well-ventilated polypropylene cages with a 12-hour light/dark cycle and a temperature between 24 and 26 °C. Before beginning treatment, the animals were given unlimited access to clean commercial food pellets and filtered tap water for a week to acclimatize to their surroundings.

Following acclimation, the mice were randomly divided into three equal experimental groups (n = 10):

- Control Group (G1): Received standard water and pellets as a vehicle control.
- BPA Group (G2): Received 400 mg/kg of Bisphenol A.
- BPA + Shilajit Group (G3): Received 400 mg/kg of Bisphenol A plus 0.5 g/kg of Shilajit.

Reagent preparation and oral dosing protocols

Bisphenol A Solution: 3 grams of pure BPA monomer were dissolved in a safe chemical vehicle made up of 25 milliliters of dimethyl sulfoxide (DMSO) and 75 milliliters of distilled water, resulting in a 25 % v/v DMSO base. A computerized magnetic stirrer was used to continuously mix the fluid at room temperature until full chemical dissolution was achieved. Final individual treatment volumes were carefully modified daily in accordance with each mouse's body weight in order to maintain a precise dosage of 400 mg/kg.

Shilajit Solution: 6 grams of pure organic Shilajit were dissolved in 30 ml of distilled water. The mixture was continuously stirred with a magnetic stirrer until a completely homogeneous aqueous solution was produced. Dosing volumes were calculated daily to ensure that each mouse received an accurate oral dose of 0.5 g/kg body weight.

All treatments were carefully delivered using specialized, smooth-tipped stainless steel oral gavage needles to ensure exact systemic absorption and minimize mechanical stress. To avoid possible compound deterioration, pure Bisphenol A and premium Shilajit were made fresh every 48 hours.

Histopathological preparation

After the 21-day course of treatment, each animal was humanely euthanized under mild anesthesia. After the thoracic cavity was surgically opened, the lung organs were carefully removed and macroscopically examined. Representative longitudinal sections of the lung tissues were obtained and promptly immersed in a 10 % neutral buffered formalin fixation solution for 48 hours in order to preserve cellular architecture. Tissue processing and slide staining were performed according to established protocols [14]. The stained lung sections were examined qualitatively under a high-resolution light microscope to evaluate structural and inflammatory changes.

Statistical analysis

Quantitative histopathological scoring and qualitative distribution data were subjected to rigorous statistical evaluation. To assess overall significance and compare differences among the three independent experimental groups, the non-parametric Kruskal-Wallis test was utilized. Following the verification of global significance,

pairwise comparisons between specific cohorts were performed using Dunn's post hoc test.

To determine the strength of the association and the dependency between the type of chemical/therapeutic exposure and the distribution of histopathological severity scores, Fisher's exact test was applied. All statistical analyses were executed using standardized computing software, and differences were considered statistically significant at a threshold of $P<0.05$.

Results and discussion

Qualitative histopathological evaluation of lung tissue

Microscopic examination of the H&E-stained lung sections across the three experimental groups revealed distinct structural variations. These findings clearly illustrate both the severe negative consequences of sub-acute Bisphenol A (BPA) exposure and the marked protective impact of concurrent Shilajit administration.

In the control group (G1), the lung tissues displayed a well-preserved, normal histological architecture. Tissue sections showed typical polygonal alveoli with thin, delicate walls distributed in regular arrays around normal terminal bronchioles. There were no signs of edema, intravascular congestion, endothelial sloughing, or inflammatory cellular infiltration. Intact pulmonary arteries maintained their normal wall thickness and structural integrity.

In contrast, the lung tissues of the BPA-exposure group (G2) exhibited extensive and severe structural damage. Microscopic analysis under a lower magnification overview revealed a significant, diffuse thickening of the interalveolar connective tissue, paired with marked architectural stress in both the respiratory parenchyma and adjacent conducting elements (***Fig. 1***).

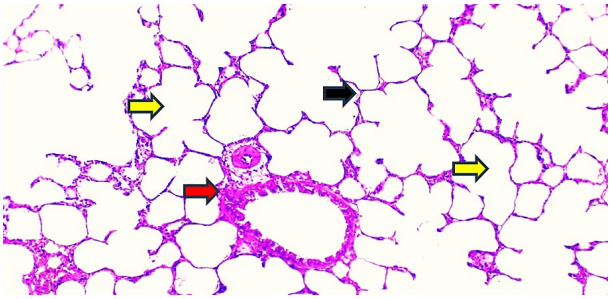

Fig. 1. Histological section of the lung from the Bisphenol A exposure group (G2) at 21 days post-exposure, revealing increased thickness of the interalveolar connective tissue due to fibrosis and mononuclear cell (MNC) infiltration (red arrow), the accumulation of proteinaceous material within the alveolar spaces (yellow arrows), and localized alveolar wall degradation (black arrow)
(H&E stain, ×100)

This low-magnification overview highlights the widespread and aggressive nature of BPA-induced pulmonary toxicity. The focal area of intense purple staining (indicated by the red arrow) marks a major zone of active chronic inflammation, where the delicate septal spaces are completely obliterated by dense sheets of mononuclear immune cells and expanding fibrotic tissue.

Concurrently, the functional gas-exchange spaces are severely compromised; the lumens of several neighboring alveoli (indicated by the yellow arrows) show an accumulation of a light-pink, amorphous proteinaceous substance, which serves as a direct indicator of increased vascular permeability and inflammatory fluid leakage. Furthermore, the structural framework of the distal lung parenchyma is highly unstable, as evidenced by micro-structural stress and early localized degradation of the fragile alveolar walls (indicated by the black arrow). These combined initial features confirm that sub-acute oral exposure to this industrial plasticizer monomer initiates rapid, multi-focal destructive processes within the respiratory tissue.

Further detailed examination of the parenchyma in the G2 group under identical magnification confirmed that the inflammatory process led to severe hemodynamic and permeability alterations. The extensive septal thickening was consistently accompanied by the formation of protein-rich fluid blocks within the respiratory spaces and structural matrices (***Fig. 2***).

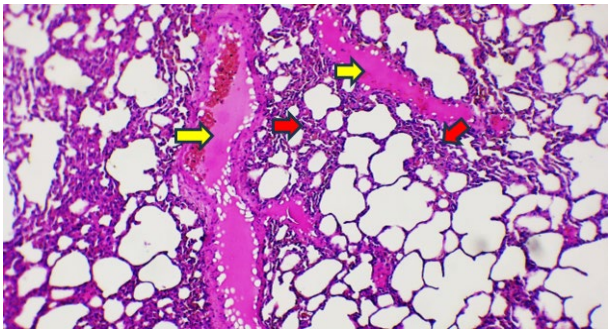

Fig. 2. Histopathological section of the lung from the Bisphenol A exposure group (G2) at 21 days post-exposure, demonstrating increased thickness of the interalveolar connective tissue due to fibrosis and mononuclear cell (MNC) infiltration (red arrows), alongside the accumulation of proteinaceous substance within the alveolar and interstitial spaces (yellow arrows)
(H&E stain, ×100)

This histopathological view highlights the severe exudative phase of BPA-induced lung injury. The large, amorphous, highly eosinophilic (bright pink) masses indicated by the yellow arrows represent dense accumulations of proteinaceous material. This material fills the alveolar gaps and invades interstitial borders, effectively blocking gas exchange.

Surrounding these exudative areas, the lung tissue loses its cellular clarity due to the heavy recruitment of immune elements; the red arrows point directly to zones of intense mononuclear cellular (MNC) infiltration and progressive interstitial fibrosis. The presence of these combined lesions indicates that sub-acute toxicity of

Bisphenol A causes severe disruption of the blood-air barrier, leading to critical fluid leakage and structural consolidation of the pulmonary tissue.

The persistent inflammatory pressure in the BPA-only treated mice also induced significant destructive changes in the conducting airways and distal parenchyma. Low-magnification overview confirmed that these lesions had a multi-focal distribution throughout the lung architecture (***Fig. 3***).

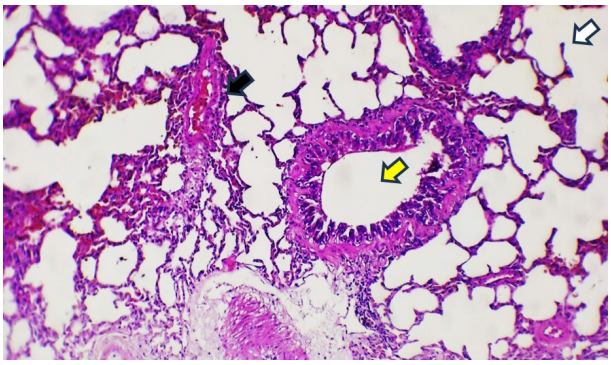

Fig. 3. Histopathological section of the lung from the Bisphenol A exposure group (G2) at 21 days post-challenge, showing perivascular leukocytic infiltration (black arrow) and epithelial desquamation of the adjacent bronchiole (yellow arrow), accompanied by the rupture of alveolar walls (white arrow)
(H&E stain, ×10)

When Shilajit was administered concurrently with BPA in the co-treatment group (G3), the pulmonary architecture was substantially preserved. Microscopic examination revealed that regular, thin-walled alveolar networks remained intact across the vast majority of the lung parenchyma, demonstrating a remarkable reduction in both septal thickening and exudative leakage.

Nevertheless, the protective efficacy, while substantial, was not entirely absolute. Localized regions within the tissue sections still displayed modest, well-contained active vascular congestion and mild perivascular mononuclear cell (MNC) infiltration.

Notably, specific areas within the interstitial alveolar fabric revealed a few well-defined, localized granulomatous accumulations. These lesions occurred concurrently with scattered, spherical micro-cavities embedded within the vascular lumens, which structurally resembled the initial stages of fat micro-embolization (***Fig. 4***).

This combined panoramic and high-power view demonstrates the localized tissue responses in the protective treatment model. The black arrow on the wider field (×100) indicates a zone where active vascular congestion and cellular infiltration remain, proving that while Shilajit efficiently prevents global tissue destruction, focal hemodynamics are still modulated.

The high-resolution inset (×400) provides critical structural evidence of specialized vascular reactions; the yellow arrow identifies clear, rounded micro-cavities within the restricted vascular tracks. These features match the morphology of early fat micro-emboli, suggesting that the co-treatment protocol triggers a localized, non-destructive intravascular lipid response during tissue recovery.

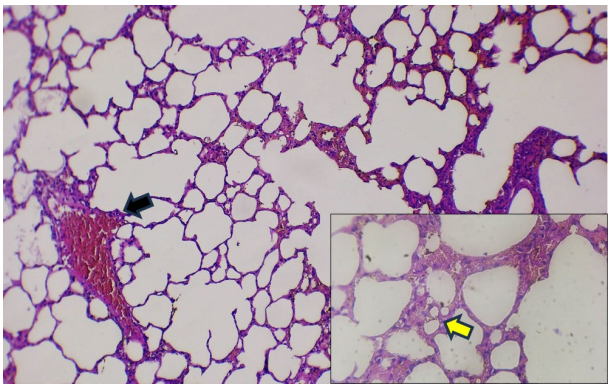

Fig. 4. Histopathological section of the lung from the Bisphenol A + Shilajit co-treatment group (G3) at 21 days post-challenge. The low-magnification background field demonstrates perivascular mononuclear cell (MNC) infiltration accompanied by vascular congestion (black arrow) (×100), while the high-magnification inset highlights microvascular changes, showing early-stage fat emboli appearing as distinctive rounded holes within the vascular spaces (yellow arrow) (×400)
(H&E stain)

Detailed screening of the pulmonary fabric under higher magnification confirmed the presence of specialized microvascular responses within the interstitial spaces. These features provide clear structural evidence of localized alterations in the distal vascular tracks (***Fig. 5***).

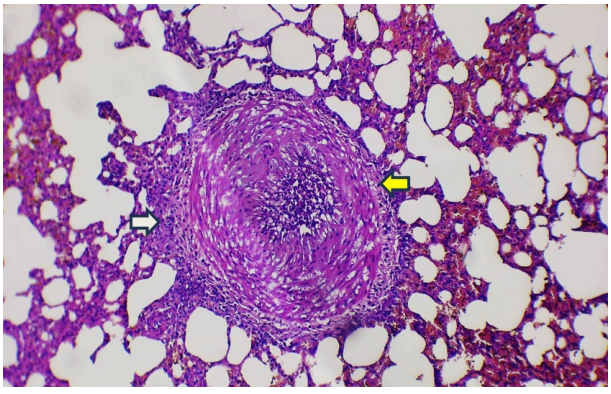

Fig. 5. High-magnification histopathological section of the lung from the Bisphenol A + Shilajit co-treatment group (G3) at 21 days post-challenge, demonstrating the formation of localized fat emboli appearing as distinctive rounded micro-cavities within the restricted vascular tracks (yellow arrow)
(H&E stain, ×400)

This microphotograph captures a critical immunomorphic feature of the Shilajit-protected lung tissue. Centrally, a large, clearly defined granulomatous accumulation occupies the interstitial space, serving as evidence of a controlled, localized immune response.

The white arrow identifies the dense surrounding zone marked by heavy perivascular mononuclear cell (MNC) infiltration and active vascular congestion, which

confines the inflammatory process and prevents its diffuse spread through the lung parenchyma. Concurrently, the yellow arrow points to the periphery of this structured focus, where clear, rounded micro-cavities indicate localized fat embolization within the microvascular beds. The structural containment of these lesions confirms that the co-treatment protocol effectively neutralizes the global destructive effects of BPA, substituting generalized parenchymal degradation with organized tissue remodeling.

Quantitative histopathological scoring and statistical analysis

The qualitative descriptive findings were fully supported by the quantitative histopathological scoring, which demonstrated substantial variations among the experimental groups. To systematically evaluate the degree of pulmonary damage, the distribution of tissue alterations was statistically analyzed across all populations (***Table 1***).

Table 1

Distribution of histopathological scores across experimental groups (% of animals)

Experimental group	Normal histoarchitecture	Minor modifications	Severe pathological changes
Control Group (G1)	90	10	0
BPA Exposure Group (G2)	10	0%	90
BPA + Shilajit Co-treatment (G3)	70	20	10

In the vehicle control group (G1), 90 % of the animals exhibited completely normal lung morphology, with only 10 % displaying minor, non-specific architectural shifts. Conversely, oral exposure to Bisphenol A in the G2 group resulted in a profound shift in tissue integrity: only 10% of the mice maintained normal architecture, while 90% developed severe, widespread histological abnormalities, confirming a statistically significant induction of targeted pulmonary toxicity.

Concurrent administration of Shilajit in the G3 group led to a remarkable structural recovery. In this co-treated cohort, the proportion of animals exhibiting normal lung histology rose significantly to 70%, while minor modifications were restricted to 20%, and severe pathological degradation was confined to just 10% of the population.

To determine the statistical significance of these distributions, a Kruskal-Wallis test was performed, revealing a highly significant overall difference among the three cohorts ($P<0.05$). Subsequent pairwise comparisons using Dunn's post hoc test confirmed that the histopathological damage scores of the BPA-exposed group (G2) were significantly elevated compared to both the control group (G1) and the Shilajit-protected group (G3). Furthermore, the Shilajit-treated group demonstrated a statistically significant improvement in structural parameters when compared directly to the BPA-only group. To explore the dependency between the therapeutic protocols and tissue outcomes, Fisher's exact test was applied, confirming a highly significant, non-random connection between the specific treatment type and the final distribution of histopathological scores ($P<0.05$).

The histopathological findings of the present study demonstrate that sub-acute oral exposure to Bisphenol A (BPA) induces severe and progressive pulmonary toxicity in a mouse model. This structural deterioration closely aligns with well-established global toxicological assessments. Comprehensive rodent bioassays managed by the National Toxicology Program have structurally documented that systemic BPA exposure leads to diffuse histiocytosis, interstitial hemorrhage, localized pneumonia, and widespread vascular congestion in mammalian lung tissues [6, 7]. Similarly, systemic challenges and low-level sub-chronic inhalation of bisphenol monomers trigger persistent inflammation of the upper respiratory architecture and localized epithelial hyperplasia, as compiled in comprehensive regulatory risk assessment reports throughout the European Union [15, 16].

In contrast, the concurrent administration of Shilajit effectively mitigated the development of these severe structural alterations, substantially preserving the primary pulmonary architecture near baseline control parameters [17]. In line with previous animal trials where variant dosages of Shilajit significantly improved weight maintenance, metabolic efficiency, and physiological growth parameters, the current investigation revealed that this traditional phytocomplex successfully maintained the animals' overall growth performance throughout the hazardous chemical exposure window [18].

This comprehensive systemic protection is directly linked to the rich abundance of essential minerals and trace elements naturally embedded within the complex organic matrices of Shilajit, particularly copper, zinc, chromium, and iron. Within modern physiological frameworks, these specific trace elements serve as crucial biological cofactors for key protective enzyme pathways – including superoxide dismutase (SOD) and catalase-thereby reinforcing the endogenous cellular defenses against foreign chemical-induced oxidative damage and subsequent parenchymal destruction [19].

Fulvic acid and humic acid represent the two primary bioactive components of Shilajit that orchestrate its multi-targeted therapeutic efficacy at the cellular level. Fulvic acid actively suppresses the basal lipid peroxidation that systematically degrades pneumocyte cell membranes. By functioning as a potent, low-molecular-weight antioxidant, it readily penetrates lipid barriers to directly neutralize harmful reactive oxygen species (ROS) within the fragile lung parenchyma [20].

Additionally, its robust anti-inflammatory dynamics are strictly mediated by the targeted suppression of pro-inflammatory cytokine pathways, specifically reducing the expression of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). This molecular down-regulation effectively minimizes severe alveolar edema and heavy leukocyte recruitment, preventing the diffuse thickening

of interalveolar septa that clinically compromises pulmonary gas exchange [21].

Conclusions

In conclusion, the present investigation demonstrates that sub-acute oral exposure to Bisphenol A (BPA) at a dose of 400 mg/kg induces profound, widespread structural degradation within the mammalian respiratory architecture, shifting the proportion of normal lung morphology down to just 10% and driving severe pathological abnormalities in 90 % of the affected population. This targeted chemical toxicity is pathomorphologically characterized by diffuse interalveolar septal thickening driven by mononuclear cell (MNC) infiltration, the accumulation of highly eosinophilic proteinaceous exudates, and progressive alveolar wall rupture that causes focal emphysema, thereby severely compromising pulmonary gas exchange. Conversely, concurrent administration of the natural mineral phytocomplex Shilajit (0.5 g/kg) exhibits a robust, statistically verified protective efficacy ($P<0.05$), successfully restoring normal lung histology in 70% of the animals and restricting severe degradation to only 10 % of the cohort. This significant structural preservation is principally driven by the synergistic antioxidant and anti-inflammatory dynamics of its core bioactive constituents – fulvic and humic acids – which down-regulate pro-inflammatory cytokine pathways (specifically TNF-α and IL-6) and mitigate lipid peroxidation across pneumocyte membranes. Furthermore, the development of well-contained interstitial granulomatous accumulations paired with localized intravascular micro-cavities (resembling early-stage fat micro-emboli) in the co-treated group underscores an active, organized tissue remodeling and defensive immune containment process. Taken together, these mathematically and histologically validated findings confirm the powerful cytoprotective potential of Shilajit, positioning it as an evidence-based natural therapeutic agent capable of safeguarding respiratory health against pervasive environmental pollutants.

DECLARATIONS

Ethical Statement

The authors confirm that all experimental procedures, husbandry protocols, and studies involving biological objects were planned and conducted in strict accordance with the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), Directive 2010/63/EU, national ethical norms, and international standards for the humane treatment of animals (NIH, ARRIVE). The research protocol and experimental scheme were officially reviewed and approved by the Scientific Ethical Committee of the College of Veterinary Medicine / University of Kerbala, Iraq (Approval No. UOK.VET.PA. 2026.182). To minimize animal stress, discomfort, and suffering, all handling, maintaining, and subsequent tissue sampling procedures involving mouse subjects were executed in strict compliance with standard veterinary guidelines, recommendations of the American Veterinary Medical Association (AVMA), and current international principles of animal welfare.

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Conflict of Interest

The author state that there is no conflict of interest.

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Declaration of AI and AI-assisted technologies

The author declares that no generative AI or AI-assisted technologies were used during the preparation, writing, translation, or language polishing of this manuscript. The final content was fully authored and reviewed by the researcher, who takes full responsibility for the integrity of the work.

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