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Therapeutic Efficacy of Novel Herbal Formulation in Protecting $PM_{2.5}$-Induced Lung Tissue Degeneration

Pooja Sharma and Era Upadhyay*

Amity Institute of Biotechnology, Amity University Rajasthan, Jaipur-303 002, India
**E-mail: era.upadhyay@gmail.com*

***Abstract*:** Particulate matter (PM) poses a major threat to human health, with lung damage evident even in early stages. Effective and low-risk treatments are essential. This study evaluated a novel herbal formulation comprising eight plants: *Angelica archangelica, Dioscorea bulbifera, Fagopyrum dibotrys, Myrtus communis, Nasturtium officinale, Perilla frutescens, Catharanthus roseus, and Solanum xanthocarpum*. The formulation was tested against PM-induced lung tissue damage in Wistar albino rats at doses of 100, 200, and 400 mg/kg over 30 days. Terminal blood samples were analyzed for hematology and differential cell count, including bronchoalveolar lavage fluid (BALF), showing significant reductions in inflammatory cells. Histopathological findings revealed PM-induced inflammation, alveolar deformation, and edema, which were progressively reduced across treatment groups, with the 400 mg/kg dose showing the greatest therapeutic effect.

***Keywords*:** Particulate matter, Lung tissue, Herbal formulation, Therapeutic actions, Wistar albino rats, Lung necropsy, Hematology

Air pollution is harmful to human health because it comprises both gaseous components and particulate matter (PM) constituents. The main components present in PM include carbonaceous material, reactive metals, nitrates, polycyclic aromatic hydrocarbons (PAHs), sulphates, endotoxins, and metals such as iron, nickel, zinc, vanadium, and copper. The primary source of PM is the combustion of fossil fuels. PM is classified based on particle size, such as PM_{10} (particles with a diameter less than 10 µm) and $PM_{2.5}$ (particles with a diameter less than 2.5 µm). Ultrafine particulate matter, referred to as $PM_{0.1}$, includes particles smaller than 0.1 µm (Hamanaka and Mutlu 2018). PM exhibits strong oxidative properties and exerts toxic effects on the human respiratory and circulatory systems. When inhaled by susceptible individuals, particulate matter triggers oxidative stress within lung cells, which contributes to the early stages of pathogenesis and poses significant risks to pulmonary health. It disrupts the balance of inflammatory cells in the lungs and stimulates the excessive production of free radicals. This overproduction, in the absence of adequate antioxidants, can lead to lung injury due to the oxidative damage inflicted on lung tissue components (Albano et al., 2022). Following PM exposure, numerous pro-inflammatory cytokines contribute to oxidative stress in lung tissues and epithelial cells, as PM acts as a potent oxidant. This leads to increased levels of inflammatory markers such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-alpha (TNF-α) in lung tissues (Chang et al., 2019). Inflammation is also characterized by elevated neutrophil counts in bronchoalveolar lavage fluid (BALF), particularly in samples from the lower respiratory tract (Clinquart et al., 2023).

Several therapeutic alternatives exist for the treatment of respiratory and pulmonary diseases. In allopathic medicine, corticosteroids such as budesonide and formoterol are commonly used but may result in side effects with prolonged use (Garg et al., 2024). In contrast, traditional medical systems including Ayurveda, Siddha, Unani, Yoga, Naturopathy, and Homeopathy, offer treatment options that are typically associated with fewer side effects and are more cost-effective. The Ayurvedic approach, which emphasizes a holistic treatment philosophy, has received increasing attention compared to allopathic treatments (Verma et al., 2024). In alignment with the 2030 Agenda for Sustainable Development, adopted by all United Nations member states in 2015, this study was conducted to formulate and evaluate the efficacy of a novel herbal formulation against particulate matter-induced lung tissue degeneration under Sustainable Development Goal (SDG) 3.9.1. The efficacy of novel herbal formulation was evaluated using complete blood count (CBC), bronchoalveolar lavage fluid (BALF) analysis, and histopathological examination of lung tissues in Wistar albino rats.

MATERIAL AND METHODS

For this study, 24 healthy adult Wistar albino rats were used. These rats were housed in an acrylic exposure chamber, and air containing particulate matter was introduced using a diesel generator. The concentration of diesel exhaust was measured using an Air Quality Monitor (GRIMM Portable Aerosol Spectrophotometer Model 11-D), which assessed PM_{10} and $PM_{2.5}$ concentrations. Lung toxicity was induced by exposing the animals to diesel exhaust for

three months, 3 hours per day. The animals were randomly assigned to four groups, with six rats in each group.

Dose administration schedule: Twenty four Wistar albino rats were divided into four groups, with six animals in each group. Group 1 (control) received distilled water orally at a dose of 5 mL/kg for 30 consecutive days, while Groups 2, 3, and 4 were administered with the test formulation orally at doses of 100, 200, and 400 mg/kg, respectively, for the same duration.

Blood sample collection and lung isolation: Blood samples were collected from the retro-orbital plexus of each animal on day 30 for complete blood count (CBC) analysis. CBC was performed using a hematology analyzer (Genrui Biotech KT-6400). Following euthanasia on day 30, the lungs were isolated for histopathological examination and bronchoalveolar lavage fluid (BALF) analysis.

Histopathological analysis: The isolated lungs were placed in buffered formalin for 24 hours. After fixation, the tissues were washed in running water for 12 hours, then processed using a tissue processor (Fig. 1). The tissue underwent a 12-step processing sequence across 12 stations, as outlined in the flow chart (Fig. 1). Tissue processing was performed to preserve structural integrity and prevent degradation. Fresh tissue was fixed in a formaldehyde solution to harden and preserve morphology. The dehydration step involved graded alcohol series in a two-step process to remove water. This was followed by clearing with acetone and benzene to extract lipids, facilitating wax infiltration. Subsequently, the tissue was infiltrated with paraffin wax. The processed tissues were embedded in L-shaped molds, filled with paraffin wax, and allowed to solidify. These tissue blocks were mounted on metal holders for sectioning. Staining was conducted as per the schedule using hematoxylin and eosin (H&E) staining (Fig. 2).

Staining procedure: Dewaxing was performed using xylene to remove residual wax. Blueing was carried out with an alkaline solution, while acid alcohol was used for differentiation, removing background staining. The sections were then stained with aqueous or alcoholic eosin, followed by rinsing, dehydration, and clearing. Finally, the slides were mounted with DPX (distrene dibutyl phthalate xylene) for microscopic examination (Bai et al., 2023).

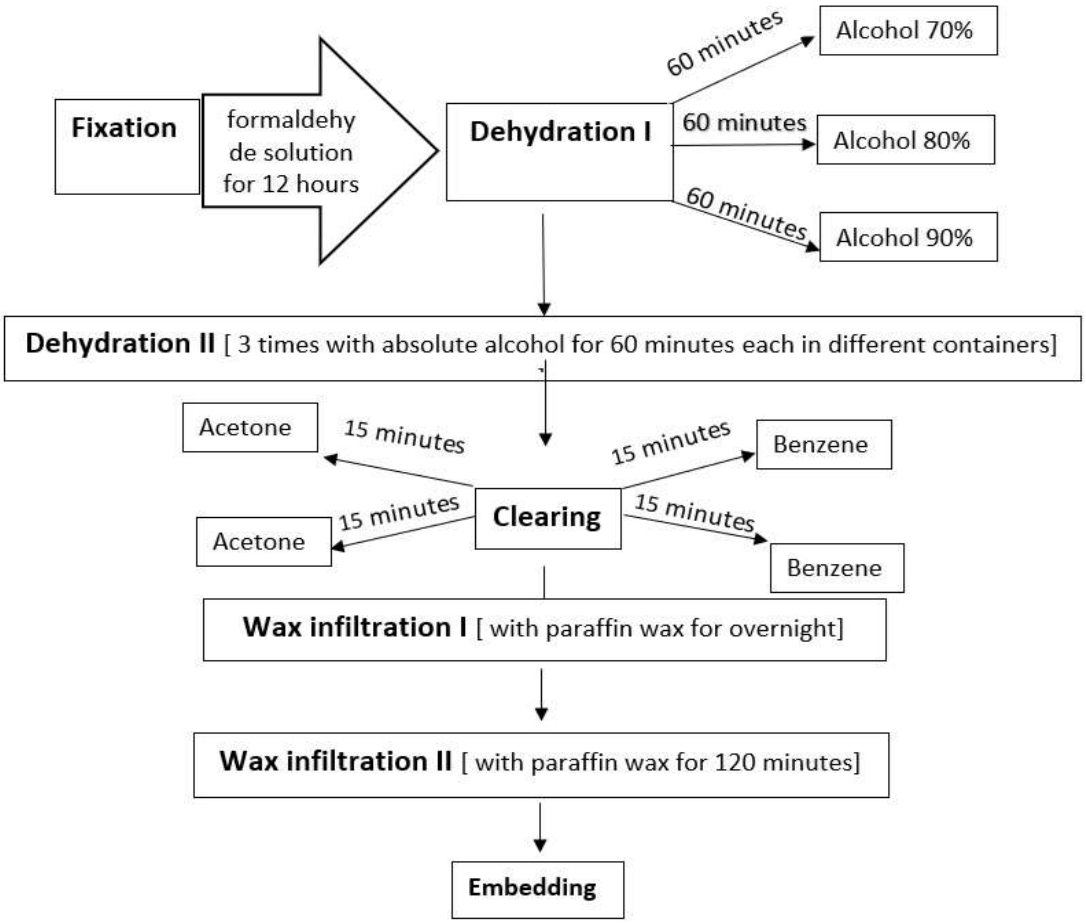

Fig. 1. Tissue processing schedule (12-step flow chart)

RESULTS AND DISCUSSION

After the 30-day dosing period, all four experimental groups were evaluated using complete blood count (CBC), leukocyte profiling in bronchoalveolar lavage fluid (BALF), and histopathological examination of lung tissues, with comparisons made relative to Group 1 (control).

Complete blood count (CBC) analysis: CBC parameters including WBC, neutrophils, eosinophils, lymphocytes, monocytes, platelets, and ESR were analyzed across groups (Table 1). No significant differences were observed in total WBC and platelet counts, suggesting that PM exposure did not markedly influence these parameters and that treatment exerted no direct effect on them. In contrast, neutrophil counts were significantly reduced in Groups 2, 3, and 4, with the maximum decrease in Group 4 (23.63%), reflecting a dose-dependent therapeutic effect. As neutrophilia is a hallmark of PM-induced pulmonary inflammation, the observed reduction suggests that the herbal formulation exerts antioxidant and anti-inflammatory activity.

Eosinophil also declined significantly in the treated groups, particularly in Group 4 (2.98%) compared with the control (6.78%), indicating a reduced requirement for eosinophil-mediated immune defense. Conversely, lymphocyte counts progressively increased from Group 2 to Group 4 (42.96% in control vs. 64.93% in Group 4), suggesting immunomodulatory effects of the formulation. Monocyte counts were moderately reduced in Group 3 and significantly reduced in Group 4, supporting attenuation of inflammatory responses. A progressive decline in ESR values across treatment groups further confirmed the suppression of systemic inflammation. Taken together, the higher lymphocyte and platelet counts in Groups 3 and 4, along with lower neutrophil, eosinophil, monocyte, and ESR levels relative to Group 1, demonstrate the dose-dependent efficacy of the formulation in restoring hematological homeostasis (Table 1).

BALF leukocyte profiling: BALF analysis was performed to directly assess pulmonary inflammation (Table 2). Neutrophil percentages were markedly reduced across treated groups, with the steepest decline observed in Group 4 (22.20%) compared to Group 1 (43.42%). Eosinophil and monocyte counts followed a similar decreasing trend, whereas lymphocyte levels increased progressively with dose, reaching 70.84% in Group 4. Statistical analysis confirmed that monocyte reductions were non-significant in Group 2, moderately significant in Group 3, and highly significant in Group 4. These findings reinforce the immunomodulatory potential of the formulation, highlighting its capacity to suppress pro-inflammatory cell infiltration while enhancing lymphocyte-mediated responses. Correlation analysis further validated the dose-dependent therapeutic effects, with Group 4 demonstrating the most consistent improvements across BALF parameters.

Histopathological examination of lung tissue: Histopathological analysis revealed distinct pathological changes in the control group (Group 1), including

Table 1. Complete blood count (CBC) parameters following 30-Day treatment across experimental groups*

Parameters	Group 1	Group 2	Group 3	Group 4
WBC (10^9/L)	4.97±0.33	5.72±0.36	5.28±0.39	4.22±0.24
Neutrophils (%)	51.04±1.66	40.31±0.57	35.96±1.22	23.63±2.52
Eosinophils (%)	6.78±0.19	5.95±0.28	4.77±0.34	2.98±0.11
Lymphocytes (%)	42.96±1.13	48.40±1.45	53.25±2.14	64.93±2.28
Monocytes (%)	3.85±0.28	3.38±0.24	2.93±0.18*	2.63±0.15
Platelet count (10^9/L)	323.33±29.71	388.00±25.18	405.00±28.52	369.50±20.86
ESR (mm/hr)	10.16±1.13	8.47±0.62	6.16±0.45	4.15±0.32

*Mean±SE

Table 2. Evaluation of leukocyte count in BALF

Parameters	Group 1	Group 2	Group 3	Group 4
Parameters	Group 1	Group 2	Group 3	Group 4
Neutrophils (%)	43.42 ± 0.94	38.54 ± 1.14 *	30.28 ± 2.38 **	22.20 ± 0.88 ****
Eosinophils (%)	9.24 ± 0.53	7.35 ± 0.29 *	5.04 ± 0.29 **	3.48 ± 0.25 ****
Lymphocytes (%)	45.45 ± 1.56	54.01 ± 1.36 *	62.94 ± 1.52 **	70.84 ± 1.10 ****
Monocytes (%)	3.94 ± 0.31	3.33 ± 0.24 ns	2.41 ± 0.12 *	2.09 ± 0.22 **

Mean±SE, Statistical significance was determined using one-way ANOVA followed by post hoc analysis. ns = not significant;*=p value <0.05, **=p value <0.01, ***= p value <0.001, ****= p value <0.0001Mean±SE , Statistical significance was determined using one-way ANOVA followed by post hoc analysis. ns = not significant;*=p value <0.05, **=p value <0.01, ***= p value <0.001, ****= p value <0.0001

inflammatory cell infiltration, alveolar distortion, edema, and thickening of intrabronchial walls, classic indicators of PM-induced lung damage. These features are closely associated with the activation of neutrophils, lymphocytes, and eosinophils, which release inflammatory mediators such as TNF-α, IL-1β, IL-4, IL-5, IL-6, histamine, leukotrienes, and nitric oxide. In contrast, Groups 2, 3, and 4 exhibited progressive histological improvements, including reduced inflammatory cell infiltration, restoration of alveolar structure, and thinning of intrabronchial walls (Fig. 3). The degree of recovery was dose-dependent, with Group 4 showing the most substantial improvements after 30 days of treatment. These findings suggest that the formulation effectively suppresses the excessive production of pro-inflammatory cytokines and reactive oxygen species (ROS), thereby protecting against PM-induced pulmonary injury and promoting structural recovery of lung tissue (Sharma and Upadhyay 2023).

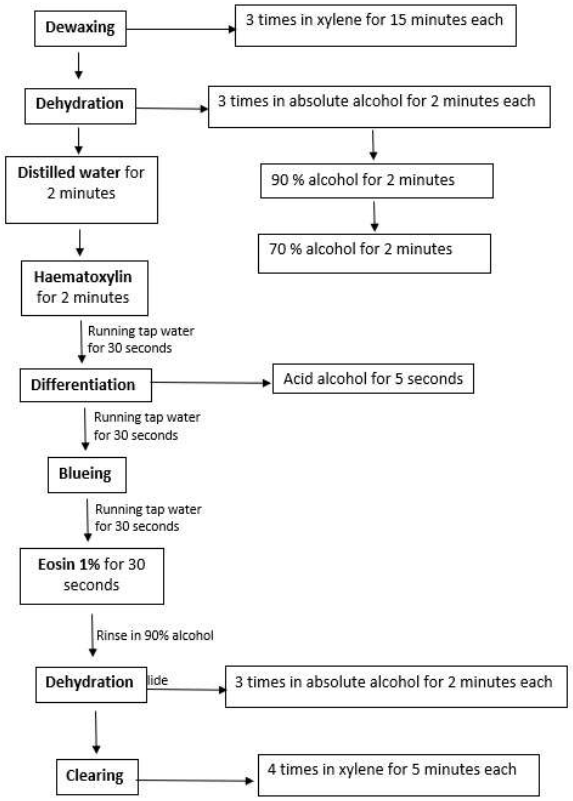

Fig. 2. Staining process schedule

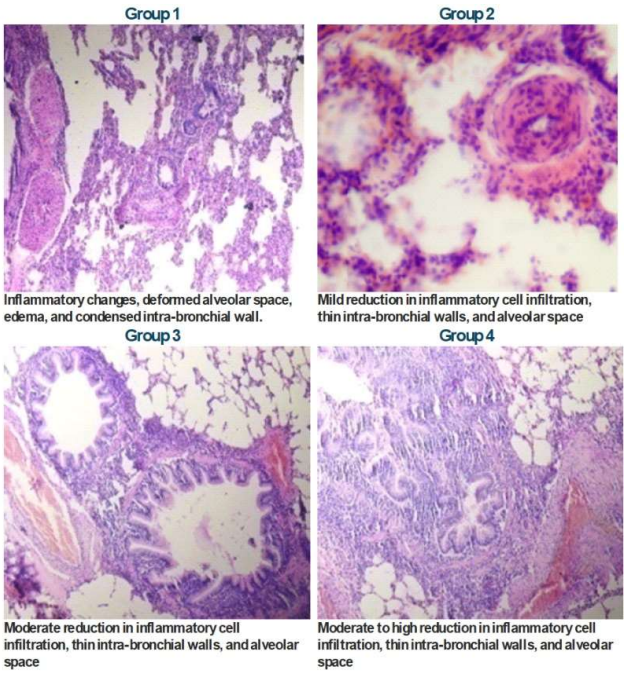

Fig. 3. Histopathological examination of lung tissues across experimental groups

CONCLUSION

Following the completion of the 30-day dosing schedule, the evaluation of hematological parameters (CBC), leukocyte profiling in bronchoalveolar lavage fluid (BALF), and histopathological examination of lung tissue served as reliable indicators of the therapeutic efficacy of the novel herbal formulation. The administered doses of 100, 200, and 400 mg/kg demonstrated significant improvements across these parameters compared to the control group.

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