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# PROTECTIVE AND RESTORATIVE EFFECTS OF *ALTHAEA OFFICINALIS* L. AQUEOUS INFUSION ON GASTRIC MUCOSAL INJURY INDUCED BY COMBINED PM2.5 AND CIGARETTE SMOKE EXPOSURE IN RATS

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## Abstract

Fine particulate matter (PM2.5) and cigarette smoke are complex inhalational toxicants capable of inducing systemic oxidative, inflammatory, vascular, and epithelial disturbances. The stomach may be particularly vulnerable because mucosal integrity depends on continuous epithelial renewal, mucus-associated defense, adequate microcirculation, and coordinated stromal support. This study evaluated the restorative potential of an aqueous infusion of Althaea officinalis L. in an experimental model of isolated and combined PM2.5 and cigarette smoke exposure. The study design included intact animals, separate exposure groups, combined exposure, and corresponding post-exposure phytotherapeutic correction groups. The infusion was prepared from pharmacopoeia-compliant dried aerial parts and roots of A. officinalis and administered orally during the corrective phase. Gastric tissue was examined using hematoxylin and eosin staining, with emphasis on epithelial integrity, glandular organization, inflammatory cellularity, stromal architecture, and the relationship between the mucosa and deeper supporting layers. Combined exposure was associated with marked disturbance of gastric mucosal organization, whereas post-exposure treatment with A. officinalis was associated with comparatively better preservation of glandular architecture, more orderly epithelial organization, lower interglandular inflammatory cellularity, and partial restoration of mucosal structural relationships. These observations are compatible with a multifactorial effect of mucilage-forming polysaccharides, flavonoids, phenolic compounds, and antioxidant constituents. The findings support further evaluation of A. officinalis as a natural restorative intervention for environmentally induced gastric injury, while targeted morphometric, biochemical, and immunohistochemical studies are required to clarify the underlying mechanisms.

**Keywords:** *Althaea officinalis*; PM2.5; cigarette smoke; gastric mucosa; mucilage polysaccharides; oxidative stress; inflammation; phytotherapy; gastric restoration.

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## Introduction

Environmental air pollution and tobacco smoke are major sources of chronic exposure to biologically active toxicants. Although their respiratory and cardiovascular effects are well established, increasing attention is being directed toward extrapulmonary organs, including the gastrointestinal tract. Fine particulate matter can trigger systemic oxidative stress, inflammatory signaling, endothelial dysfunction, mitochondrial injury, and disturbances of cellular homeostasis, mechanisms that are increasingly implicated in digestive disease [1].

The stomach is potentially susceptible to these effects because gastric mucosal homeostasis depends on a tightly coordinated interaction among surface and glandular epithelium, mucus-associated protection, local microcirculation, immune cells, and stromal support. Disruption of any of these components may impair epithelial restitution and weaken resistance to additional luminal or systemic stressors.

Cigarette smoke adds a second complex toxicological burden. Its constituents include reactive oxidants, nicotine, aldehydes, and numerous other chemicals capable of modifying mucosal perfusion and tissue repair. Experimental studies have shown that tobacco smoke can aggravate gastric mucosal injury, attenuate injury-associated hyperemia, increase epithelial apoptosis, and delay ulcer healing [2-4]. These observations provide a biological rationale for considering combined pollutant exposure as a potentially stronger challenge to gastric homeostasis than either exposure alone.

Natural products with barrier-supporting, antioxidant, and anti-inflammatory properties may be useful in the recovery phase after toxic exposure. Althaea officinalis L. (Malvaceae) is a medicinal plant traditionally associated with soothing and mucosal-protective applications. Its roots and aerial parts contain mucilage-forming polysaccharides together with flavonoids, phenolic compounds, and other bioactive constituents. Polysaccharides are considered among the major pharmacologically relevant components of the plant and have been associated with antioxidant, wound-healing, and immunomodulatory activities [5].

Experimental evidence also supports the gastric relevance of A. officinalis. Previous studies of aqueous preparations and plant extracts have described anti-inflammatory and gastroprotective actions in experimental models [6,7]. However, conventional studies have largely focused on chemically induced gastric damage or prophylactic treatment. The restorative potential of A. officinalis after combined inhalational exposure to PM2.5 and cigarette smoke remains insufficiently characterized.

The present study therefore evaluated the gastric morphological response to isolated and combined PM2.5 and cigarette smoke exposure and examined whether post-exposure administration of A. officinalis aqueous infusion was associated with restoration of mucosal architecture. Particular attention was given to the epithelial-glandular compartment, interglandular stroma, inflammatory cellularity, and the relationship between the mucosa and deeper supporting structures.

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## Materials and Methods

### *Experimental Design*

Adult white laboratory rats were maintained under standard experimental conditions and allocated according to the type of inhalational exposure and subsequent phytotherapeutic correction. The design included seven experimental groups and allowed assessment of isolated pollutant effects, combined exposure, and post-exposure correction. The principal comparison for the present article was between Group 4, representing combined PM2.5 and cigarette smoke exposure, and Group 7, in which the same combined exposure was followed by Althaea officinalis L. aqueous infusion.

**Table 1. Experimental groups.**

| Group | Experimental condition |
|---|---|
| Group 1 - Control | Intact animals without experimental exposure. |
| Group 2 - PM2.5 | Animals exposed to PM2.5 by inhalation. |
| Group 3 - Cigarette smoke | Animals exposed to cigarette smoke by inhalation. |
| Group 4 - PM2.5 + cigarette smoke | Animals subjected to combined inhalational exposure to PM2.5 and cigarette smoke. |
| Group 5 - PM2.5 + correction | Animals receiving Althaea officinalis L. aqueous infusion after PM2.5 exposure. |
| Group 6 - Cigarette smoke + correction | Animals receiving Althaea officinalis L. aqueous infusion after cigarette smoke exposure. |
| Group 7 - PM2.5 + cigarette smoke + correction | Animals receiving Althaea officinalis L. aqueous infusion after combined PM2.5 and cigarette smoke exposure. |

### *Inhalational Exposure Model*

PM2.5 and cigarette smoke exposures were carried out using the established inhalational chamber protocol. Separate exposure groups received the individual toxicants, whereas the combined-exposure groups were subjected to both factors. The model was designed to reproduce repeated inhalational exposure and to assess the gastric response to overlapping environmental toxicants. Because the present manuscript emphasizes morphological interpretation rather than quantitative exposure-response analysis, the exposure conditions are described here according to the experimental grouping and should be accompanied by the full chamber parameters in the final submission record.

### *Plant Material and Preparation of the Aqueous Infusion*

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The corrective preparation consisted of an aqueous infusion of Althaea officinalis L. prepared from pharmacopoeia-compliant dried aerial parts and roots. Before extraction, the plant material was pulverized. Ten grams of dried plant material were added to 100 mL of boiling distilled water, maintained in a water bath for 15-20 min, cooled at room temperature for 45 min, and filtered through two layers of sterile gauze. The infusion was prepared fresh for administration. This aqueous preparation was selected to favor extraction of hydrophilic constituents, particularly mucilage-forming polysaccharides and other water-soluble components relevant to mucosal protection.

### *Phytotherapeutic Correction*

The freshly prepared infusion was administered orally by gastric gavage after completion of the experimental inhalational exposure. The dose was calculated according to body weight and corresponded to 100 mg/kg as a crude-plant equivalent. Because treatment followed the toxic exposure period, the intervention was interpreted primarily as a corrective and restorative approach rather than prophylactic pretreatment.

### *Histological Processing and Morphological Assessment*

At the experimental endpoint, gastric tissue samples were collected and processed using standard paraffin histology. Sections were stained with hematoxylin and eosin (H&E) and examined by light microscopy. Morphological assessment focused on epithelial integrity, glandular organization, cytoplasmic dystrophic and vacuolar changes, inflammatory cellularity within the lamina propria, interglandular stromal organization, and the relationship between the mucosa and deeper supporting layers. Particular attention was given to direct comparison of Group 4 and Group 7.

### Interpretive Approach

The current manuscript presents the histological component in a qualitative, morphology-centered format. No numerical morphometric effect sizes are introduced in order to avoid overinterpretation before completion of the quantitative dataset. Terms such as “improved organization” and “partial restoration” therefore refer to comparative histological patterns visible in representative H&E sections and do not imply complete normalization or a quantified treatment effect.

## Results

### Gastric Morphology after Combined PM2.5 and Cigarette Smoke Exposure

The combined-exposure group showed the most conspicuous disturbance of normal gastric mucosal organization among the specimens emphasized in the present article. Representative H&E sections demonstrated irregular and heterogeneous glandular profiles, disruption of orderly epithelial arrangement, cytoplasmic vacuolar and dystrophic changes, and increased cellularity within interglandular tissue. The relationship between adjacent glands and the surrounding stromal compartment appeared less regular, producing a

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heterogeneous mucosal architecture. These features indicate that the combined insult involved the epithelial-glandular unit together with its supporting interglandular compartment rather than isolated epithelial changes alone (Figure 1).

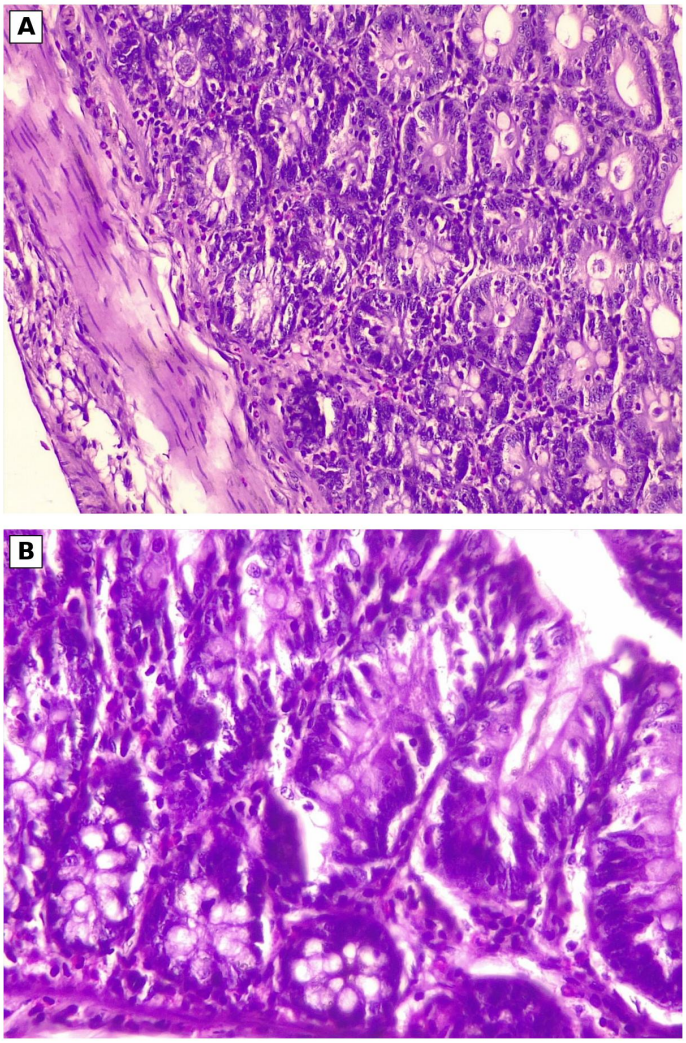



**Figure 1. Histopathological alterations of the gastric mucosa following combined inhalational exposure to PM2.5 and cigarette smoke (Group 4).** (A) Representative H&E-stained section demonstrating marked disturbance of glandular architecture, epithelial dystrophic changes, increased interglandular cellularity, and disorganization of the mucosal stromal compartment. (B) Higher-magnification field demonstrating epithelial disarray, cytoplasmic vacuolar change, irregular glandular profiles, and inflammatory cellularity within the interglandular tissue. Hematoxylin and eosin staining.

**Gastric Morphology after Correction with Althaea officinalis L.**

In the post-exposure correction group, the histological pattern was comparatively more organized. Gastric glandular profiles were more regularly arranged, epithelial architecture appeared more coherent, and conspicuous interglandular cellularity was less prominent

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than in the untreated combined-exposure group. In broader fields, the mucosal compartment showed improved continuity and a more orderly relationship with deeper supporting tissue. Residual heterogeneity remained, and the pattern is therefore more appropriately interpreted as partial structural restoration rather than complete normalization (Figure 2).

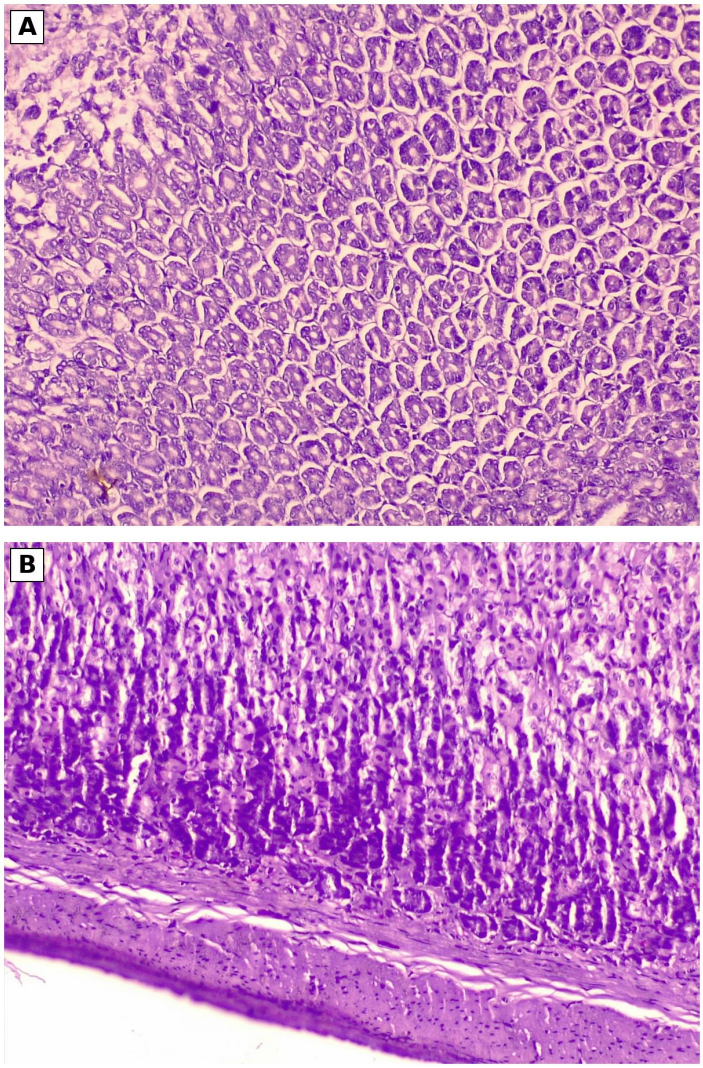



**Figure 2. Gastric mucosal morphology following correction with Althaea officinalis L. aqueous infusion after combined PM2.5 and cigarette smoke exposure (Group 7).** (A) Representative H&E-stained section showing comparatively improved preservation and regularity of glandular structures, more orderly epithelial organization, and less prominent interglandular inflammatory cellularity than in Group 4. (B) Broader field demonstrating partial restoration of mucosal architecture and a comparatively more organized relationship between glandular structures and deeper supporting layers. Hematoxylin and eosin staining.

***Comparative Morphological Interpretation of Groups 4 and 7***

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Direct comparison of Groups 4 and 7 suggests that the principal morphological difference after phytotherapeutic correction involved the integrity of the epithelial-glandular-stromal unit. The untreated combined-exposure specimens showed greater architectural disorganization and inflammatory cellularity, whereas corrected specimens demonstrated comparatively better tissue organization and structural continuity. The available H&E material supports a restorative interpretation but does not establish the magnitude of recovery or a specific molecular mechanism.

## Discussion

The present study evaluated gastric injury associated with combined PM2.5 and cigarette smoke exposure and examined the restorative potential of Althaea officinalis L. aqueous infusion. The findings are consistent with the concept that inhaled environmental toxicants can affect the gastrointestinal tract through systemic oxidative, inflammatory, vascular, and epithelial mechanisms.

PM2.5 is increasingly recognized as an extrapulmonary toxicant capable of disrupting redox balance, inflammatory regulation, endothelial function, and cellular homeostasis [1]. These effects are particularly relevant to the stomach, where mucosal integrity depends on continuous epithelial renewal, adequate perfusion, and coordinated interactions among epithelial, stromal, immune, and vascular components. Cigarette smoke may intensify these disturbances through oxidants, nicotine, aldehydes, and other toxic constituents that impair mucosal defense and local circulation. Experimental work has shown that tobacco smoke can aggravate gastric mucosal injury, attenuate protective hyperemia, increase apoptosis, and delay healing [2-4]. Their combined action may therefore create overlapping pathways of gastric injury, although the degree of interaction between the two exposures remains to be clarified.

Against this background, the use of A. officinalis as a post-exposure intervention is especially relevant. Unlike prophylactic models, the present design focuses on recovery after toxic injury has already developed. Accordingly, the observed tissue changes should be interpreted in the context of epithelial restitution, stromal reorganization, and restoration of mucosal homeostasis rather than simple prevention of damage.

The biological plausibility of this restorative effect is supported by the phytochemical profile of A. officinalis. The plant contains mucilage-forming polysaccharides, flavonoids, phenolic compounds, and other bioactive constituents that may act through complementary mechanisms. Polysaccharides are among the major characterized bioactive fractions of A. officinalis and have been associated with antioxidant, wound-healing, and immunomodulatory activities [5]. Their hydrophilic and viscoelastic properties also provide a plausible basis for local mucosal barrier support. Because mucus production and mucin expression were not directly assessed, however, a barrier-supporting action should be considered biologically plausible rather than demonstrated.

Flavonoids and phenolic constituents may provide an additional antioxidant component. Oxidative stress is a common consequence of both particulate pollution and cigarette smoke exposure and can contribute to membrane damage, mitochondrial

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dysfunction, inflammatory activation, and impaired regeneration. Previous pharmacological studies have reported antioxidant and gastroprotective effects of A. officinalis preparations in experimental gastric injury [6,7]. These reports are consistent with the direction of the present morphological observations, although differences in model design preclude direct equivalence.

Inflammatory modulation may represent another component of the restorative response. Persistent inflammatory activation can prolong gastric injury through cytokine release, reactive metabolites, and proteolytic mediators. The histological pattern after A. officinalis treatment is compatible with attenuation of this secondary inflammatory component, but morphology alone cannot determine the signaling pathways responsible. Direct assessment of inflammatory mediators would therefore be required to confirm this interpretation.

The recovery process may also extend beyond the epithelial compartment. Gastric mucosal integrity depends on stromal organization, extracellular matrix support, and adequate microvascular function. Thus, a more organized relationship between glandular and supporting tissues after correction may reflect a broader restoration of mucosal architecture rather than an isolated epithelial response. This consideration is particularly relevant to pollutant-associated injury, in which vascular and stromal dysfunction may contribute to delayed epithelial recovery.

These observations provide a rationale for further mechanistic studies. The balance between Nrf2-mediated antioxidant defense and NF-kB-dependent inflammatory signaling may be particularly informative, while COX-2, Caspase-3, and Ki-67 could help characterize inflammatory, apoptotic, and regenerative responses. Assessment of MUC1 and MUC5AC would also clarify whether A. officinalis directly influences mucosal barrier function. Such analyses should be viewed as future mechanistic extensions rather than inferred outcomes of the present H&E findings.

The main novelty of the model lies in combining environmental toxicology with phytotherapeutic restoration. In contrast to conventional chemically induced gastric injury models, the present study examines gastric alterations following inhalational exposure to two environmentally relevant toxicants and evaluates recovery after exposure has ceased. This design may provide a useful framework for studying natural restorative agents under conditions that more closely approximate complex environmental exposure.

The study has limitations. The aqueous infusion was not subjected to quantitative phytochemical profiling, and the current interpretation is based primarily on morphological findings together with the known composition of A. officinalis. Future work should therefore include standardized characterization of the preparation, quantitative morphometry, oxidative stress biomarkers, inflammatory mediators, and immunohistochemical analysis.

Overall, the findings support the hypothesis that A. officinalis aqueous infusion may facilitate partial recovery of gastric tissue after combined PM2.5 and cigarette smoke exposure. This effect may involve complementary support of the mucosal barrier, antioxidant defense, inflammatory control, and epithelial-stromal restoration, although direct biochemical and molecular confirmation is still required.

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## Conclusion

Combined PM2.5 and cigarette smoke exposure was associated with marked disturbance of gastric mucosal architecture involving epithelial, glandular, and stromal components. Post-exposure administration of Althaea officinalis L. aqueous infusion was associated with comparatively better preservation of glandular organization, more orderly epithelial structure, less prominent inflammatory cellularity, and partial restoration of mucosal tissue relationships. The effect is biologically compatible with the combined actions of mucilage-forming polysaccharides, flavonoids, phenolic constituents, and other antioxidant or anti-inflammatory components of the plant. The present findings support further evaluation of A. officinalis as a natural restorative intervention in environmentally induced gastric injury, while quantitative morphometry, phytochemical standardization, and targeted molecular studies are required for mechanistic confirmation.

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