Preprints.org

Article

Not peer-reviewed version

Temporal Effects of Cigarette Smoke and Phytochemical-Based E-Liquid Aerosols on Tracheo-Alveolar Histopathology and the IL-6/TNF-α Molecular Signaling Axis

Awal Prasetyo *, Dora Maftikhati, Levina Athaya Anarizta, Nazhira Ghina Setyawan, Anindha Waradita Putri Yuwono, Maria Meutia Saleha, Farahdita Ramadhanti, Hermawan Istiadi, Udadi Sadhana, Fathur Nur Kholis

Posted Date: 18 May 2026

doi: 10.20944/preprints202605.1119.v1

Keywords: cigarette smoke; electronic nicotine delivery; ascorbic acid; IL-6; TNF-α; SOD-3; MDA; tracheo-alveolar histopathology

Preprints.org is a free multidisciplinary platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC, OpenAlex.

Copyright: This open access article is published under a Creative Commons CC BY 4.0 license, which permit the free download, distribution, and reuse, provided that the author and preprint are cited in any reuse.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 May 2026 doi:10.20944/preprints202605.1119.v1

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Article

Temporal Effects of Cigarette Smoke and Phytochemical-Based E-Liquid Aerosols on Tracheo-Alveolar Histopathology and the IL-6/TNF-α Molecular Signaling Axis

Awal Prasetyo [1,2,*], Dora Maftikhati [1], Levina Athaya Anarizta [1], Nazhira Ghina Setyawan [3], Anindha Waradita Putri Yuwono [3], Maria Meutia Saleha [3], Farahdita Ramadhanti [3], Hermawan Istiadi [2], Udadi Sadhana [2] and Fathur Nur Kholis [4]

[1] Department of Biomedical Science, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia
[2] Department of Anatomic Pathology, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia
[3] Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia
[4] Division of Pulmonology, Kariadi Hospital, Department of Internal Medicine, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia
* Correspondence: awalpras@fk.undip.ac.id; Tel.: +62-08122810954

Abstract

This study investigates the comparative effects of traditional cigarettes and e-cigarettes on lung health in male *Rattus norvegicus* over 8 and 12 weeks. Following ARRIVE 2.0 guidelines, 30 rats were divided into six groups to evaluate the impact of nicotine and ascorbic acid aerosols on tracheal and alveolar structures, as well as systemic inflammatory markers (IL-6, TNF-α, SOD-3, MDA). Results indicate that long-term cigarette exposure (12 weeks) and nicotine vaping (8 weeks) significantly stunted weight gain, whereas ascorbic acid vaping caused less growth inhibition. Histological analysis revealed that 8-week cigarette exposure (K3) increased tracheal mucosal thickness and antioxidant activity (SOD-3), while cigarette smoke generally decreased goblet cell counts and induced early emphysema. In contrast, long-term exposure significantly elevated IL-6 and caused severe alveolar wall damage. Notably, vaping ascorbic acid (K6) offered protective benefits, preserving the basement membrane and reducing septal thickening compared to nicotine groups. The findings conclude that while short-term smoking triggers immediate tracheal damage, long-term exposure escalates systemic inflammation and permanent alveolar destruction. Phytochemical-based aerosols, such as ascorbic acid, reduce pulmonary injury compared with nicotine-based products.

Keywords: cigarette smoke; electronic nicotine delivery; ascorbic acid; IL-6; TNF-α; SOD-3; MDA; tracheo-alveolar histopathology

1. Introduction

The rising number of smokers is a global challenge. Smoking devices have become more varied, including traditional cigarettes and electronic cigarettes (e-cigs), aerosol, commonly referred to as vapes. In 2020, about 1.18 billion people smoked tobacco [[1]], while an estimated 68 million used vapes [0,2] and an additional 82 million worldwide in 2021 [3]. Both smoking and vaping cause serious issues like lung damage, heart disease, and addiction. Vapes release harmful substances, including nicotine, tiny particles, heavy metals, and chemicals, and are a dangerous alternative to cigarettes [4,5,6]. Other harmful substances are present in e-liquids heated with humectants, flavorings, propylene glycol, glycerin, and aldehyde, which become aerosolized and later cause notable harm [7,8]. Smoking, whether through cigarettes or vapes, can lead to long-term health

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 May 2026 doi:10.20944/preprints202605.1119.v1

issues, including increased oxidative stress, airway inflammation, immune suppression, and impaired alveolarization [9,10].

E-cigarette aerosol harms the trachea and lungs by causing inflammation and damaging the airway. Small particles penetrate deep into the lungs, while larger ones settle in the trachea. This injury leads to significant lung changes due to chemical damage and inflammation. Key effects include the accumulation of lipid-laden macrophages, the death of lung cells, and increased neutrophilic inflammation. As a result, the alveolar walls become thicker, the structure of the alveoli is damaged, and more inflammatory cells invade the area [11,12]. Ingredients like propylene glycol and vegetable glycerin dry out the respiratory lining, making it harder to clear mucus. High wattage in heating e-liquids creates harmful chemicals that damage cell membranes and weaken airway function. Both e-cigarette aerosol and cigarette smoke can harm the trachea and alveoli through inflammation and cell toxicity [13,14].

The aerosol weakens connections between epithelial cells in the airways, damaging the airway surface. Toxic substances kill these cells, leading to shedding and exposing the underlying layer. This injury causes inflammation, as damaged cells release signals such as IL-1β, IL-6, and IL-8, which attract neutrophils and macrophages. These produce Tumor Necrosis Factor-alpha (TNF-α) and reactive oxygen species (ROS), causing more inflammation. Levels of pro-inflammatory cytokines like IL-6 and TNF-α rise in the lungs. Studies show that even short-term exposure to flavored e-cigarette vapor can cause lung inflammation, while longer exposure can maintain this inflammation, harm lung structure, and reduce lung function. E-cigarette chemicals also irritate the trachea and worsen inflammation [15,16,17].

The “harm reduction paradox” of e-cigarettes highlights a conflict: they can help smokers quit burning tobacco and reduce harm, but they may also create a new generation of nicotine-addicted young users who might not have smoked otherwise, which increases harm [18]. In addition, many people think that using ascorbic acid (Vitamin C) or fruit-flavored liquids is a healthier alternative to smoking. These products claim to provide a "wellness" experience and antioxidant benefits, serving as a safe way to satisfy the hand-to-mouth habit without nicotine addiction [19]. The hypothesis that breathing in ascorbic acid (AA) may support the immune system in the nose and sinuses, and might also reduce inflammation, especially in individuals with asthma or breathing problems [20], has to be proved. The antioxidant property of AA could reduce the amount of ROS in the body and prevent lipid peroxidation through electron transfer [21,22], but it may not have the same effect in the form of vapors, and the research on the safety and toxicity of inhaling AA is limited. This presumption is largely unproven, with significant research indicating potential risks rather than health benefits, and creating a complex public health challenge [23].

Common markers for assessing oxidative stress were superoxide dismutase-3 (SOD-3) and malondialdehyde (MDA). SOD-3 is an extracellular enzyme that can suppress the inflammatory response. SOD-3 has been shown to maintain ECM structure, reduce oxidative stress in macrophages, and increase lung space [24,25]. MDA is a lipid peroxide marker used to assess oxidative stress and redox signaling. Elevated MDA levels indicate increased lipid peroxidation. Lipid peroxidation is highly linked to the amount of ROS, whereas lipid peroxidation is susceptible to oxidation by free radicals. MDA is considered a reliable marker of oxidative stress due to its reactivity and toxicity [26,27,28,29].

The microscopic tracheal damage caused by chemical aerosol agents, including desquamation, reduced cilia, and increased goblet cells, leads to mucosal hypersecretion. It would eventually disrupt the nasal mucociliary (NMC) mechanism, an innate defense that protects the respiratory tract from pathogens, consisting of the mucous layer, airway surface liquid layer, and ciliary epithelium. Harmful ingredients, both in vapes and cigarettes, have been proven to affect tracheal histology. The tracheal mucus entraps vape and/or cigarette smoke, later triggering the proliferation of goblet cells. Increasing goblet cells would elevate mucin expression, thereby increasing mucosal thickness. The aforementioned alterations in the trachea would subsequently decrease the effectiveness of NMC [30].

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 May 2026 doi:10.20944/preprints202605.1119.v1

Smoking, whether through cigarettes or vapes, harms the body in different ways. Nicotine is a common e-liquid ingredient, and researchers are exploring the use of vitamins as alternatives [31], and the different duration of exposure. This study will compare different exposure times and the effects of nicotine and AA in vaping versus cigarette smoke. It will measure plasma levels of proinflammatory cytokines (IL-6, TNF-α), oxidative stress markers (SOD-3, MDA), goblet cell counts, tracheal epithelial thickness, and alveolar-pulmonary changes.

2. Materials and Methods

In order to reach a transparent and accurate reporting of research, this study used the ARRIVE guidelines 2.0 (Animal Research: Reporting In Vivo Experiments) [32].

2.1. Study Design

This is a true experimental randomized posttest-only control group design that rigorously tests causality by randomly assigning subjects to experimental or control groups, introducing an intervention (cigarette smoke and aerosol vapor exposure as independent variable) only to the experimental group, and measuring outcomes (proinflammatory cytokines, oxidative stress, and trachea-alveolar histopathology as dependent variables) for both groups solely after the intervention, ensuring high internal validity.

2.2. Sample Size

A total of 30 rats were used as subjects; each experimental unit was allocated 5 rats, and the minimal sample size was revealed using the "resource equation" method [33] because the effect size or standard deviation is unknown. It relies on the degrees of freedom *(df)* for the error term in an Analysis of Variance with the value of $E = Total\ number\ of\ animals - Total\ number\ of\ groups$. This study examined the effect of an independent variable and included 6 groups (4 treatments and 2 controls), with 5 rats per group. Total subjects per group in this case: E = 30 – 6= 24, which is within the acceptable limit and hence an adequate sample size. With E = 24, the total sample size is robust and generally considered highly acceptable in molecular signaling research (IL-6/TNF-α), where biological variability can be high or "drop-outs" during the smoke-exposure phase might occur. The probability of detecting an effect if one exists for "all-or-nothing" or "large" morphological changes in tracheo-alveolar tissue with n=5 per group was "Power" (1 - β), achieving a power of approximately 80%, assuming a large effect size (d ≥1.5).

2.3. Inclusion and Exclusion Criteria

The animals were included in the study if they successfully underwent the intervention. Rats with excessive weight loss exceeding 20% of initial weight after acclimation, any underlying sickness, development of motor impairments that could affect behavioral measurements, or unexpected injury will be excluded. Rats that died during the research period, from acclimatization through termination, were dropped out.

2.4. Grouping and Randomization

All rats were divided into control groups without any exposure, consisting of two durations: 8 and 12 weeks (K1 and K2). The four other treatment groups had different durations and aerosol exposures: three cigarettes smoked/day for 8 weeks (K3) and 12 weeks (K4); e-nicotine liquid-based vape aerosol for 8 weeks (K5); and e-ascorbic acid liquid-based vape aerosol for 12 weeks (K6).

Simple random sampling was used to allocate subjects to control and treatment groups by creating a numbered list of all rats and selecting subjects at random. Every subject has an equal chance of selection, ensuring an unbiased subset, usually achieved via lottery methods. To minimize confounders (treatment order or housing location), we randomized treatments and measurements, counterbalanced, and blocked. In each treatment schedule and measurement, the experimental units

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 May 2026 doi:10.20944/preprints202605.1119.v1

and subjects were randomized daily to prevent time-of-day effects and were randomly assigned positions on a rack to control for environmental variations (light/temp).

Before and after a week of acclimatization, all subjects were weighed to ensure that each treatment group was balanced for body weight, controlling for potential confounding differences. All procedures (weighing, aerosol exposure, and blood sampling) were conducted by the same person, or the investigators were blinded to treatment groups during testing to prevent observation bias.

2.5. Blinding

The animal caretakers and data analysts were blinded to the stages (i.e., allocation, intervention, or outcome assessment) and were unaware of group assignments, which were coded to prevent conscious or subconscious bias in data collection and analysis.

2.6. Statistical Methods

Statistical analysis was performed using IBM SPSS Statistics 25, employing a one-way analysis of variance (ANOVA) followed by Tukey's HSD to assess differences between experimental groups. This specific combination of tests is used to compare the averages of three or more groups to see the differences of histopathological changes in the trachea-mucosa and alveolus, as well as IL-6 and TNF-α, and SOD-3 and MDA levels, as dependent variables measured over time in the same subjects, controlling for type I error inflation while identifying specific differences.

2.7. Experimental Animals

A total of 30 male *Rattus norvegicus*, 8-week-old and weighing 160-180 g, were obtained and bred specifically for scientific purposes in the Biology Laboratory at Semarang State University. Otherwise, housing and rat treatment were conducted at the Animal Laboratory of the Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia.

2.8. Acclimatization

All rats in five at each experimental group were maintained in a stable room with a 12-h light-dark cycle, temperatures between 22-26°C, an appropriate humidity of 40-60%, and a stimulating, clean, spacious environment with dim, indirect light and good air circulation, but no direct draught. For one week, the rats underwent an acclimatization period and were given standard feed A594K pellet and water ad libitum. All subjects were weighed using a pharmacologic digital scale (0.1-5000 g) before treatment.

2.9. Experimental Procedures

A Cigarette smoke-chamber and Electronic-cigarette-vapor chamber made to order by custom, and following the protocol of the University of Michigan Institutional Animal Care and Use Committee (IACUC) [34].

2.9.1. Cigarette Smoke Exposure

The local filtered clove cigarettes (kretek) were used to simulate smoking exposure in 18-liter, whole-body, semi-closed exposure chambers, which are commonly used to mimic second-hand smoke exposure and feature controlled air inlets and outlets to maintain a consistent smoke concentration. The smoke was pushed into the chamber using "syringe method" (a manually replicated "puff" action of a human smoker) with a basic manifold to connect the cigarette to the chamber: the "holder" (a rubber stopper or plastic tube that fits the cigarette filter tightly), the "puff" tool (a large 50 mL plastic syringe), three-way stopcock (allows to draw smoke from the cigarette and then switch the path to push it into the box without disconnecting everything), and inlet port (a small hole in your container box (sealed with a grommet) where the smoke enters).

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 May 2026 doi:10.20944/preprints202605.1119.v1

The exposure time is based on a typical inhalation protocol that involves exposing the rats to the smoke of 1.25 cigarettes over 30 minutes, twice a day, with a 5-minute ventilation period in between to prevent hypoxia. The dosage of cigarettes that is used in this research is equivalent to the dosage of heavy smoking in humans, which is generally defined as smoking 20 or more cigarettes per day (approximately one pack or more). To calculate the equivalent dose for a rat based on human cigarette consumption using the Laurence & Bacharach method, a body-weight-to-surface-area ratio is typically used, with the standard conversion factor from a human (70 kg BW) to a rat (200 g) of 0.018. For adjusting for the larger volume of an 18-liter container, here is the calculation for 6 rats (180 g each) to match the exposure of a human smoking 20 cigarettes per day: 0.324 cigarettes per rat, and the total biological requirement for 6 rats was 1.944 cigarettes per day, or 2.33 cigarettes per container per day, or the same as 3 cigarettes for equalization.

2.9.2. Electronic-Vape Aerosol Exposure

An electronic-vape aerosol exposure technique was performed manually to investigate the impact of e-cigarette aerosol (nicotine-liquid base and ascorbic-acid liquid base) or to measure secondhand exposure in environmental simulations on the tracheal-alveolar system. These techniques involve a controlled, non-automated vape mod to generate aerosol from nicotine or ascorbic acid-containing e-liquid, which is then delivered to an exposure chamber. The dosage for nicotine e-liquid-based vape aerosol equivalent to high-intensity toxicological dose [35], using a 0.5 mL daily 0.5 ml/day every day with puffs lasting 4 seconds at 30 second intervals, intake for a 200g rat to make effective "stresses" the system significantly, equal with maximum human use, ensuring that any potential respiratory or vascular damage becomes visible in a short-duration (8 weeks) study. The ascorbic acid dose is 0.5 ml/day (100 mg), which was also administered to a treatment group and is approximately equivalent to 4.8 g/day in humans. This is significantly equal to the standard human dietary maximum (2 g) and would be considered a pharmacological or high-dose experimental level.

2.9.3. Euthanasia

At the end of 8 and 12 weeks, rats were properly euthanized using humane endpoints, and all procedures followed AVMA guidelines. In this research setting, the decapitation technique is performed by trained personnel using well-maintained equipment to preserve cellular stability for accurate measurement of oxidant and antioxidant enzymes.

2.9.4. Preparation of Trachea-Alveolar Specimen for Histological Examination

Harvesting the trachea of a rat involves careful dissection of the cervical region to isolate the airway from surrounding connective tissue and muscles. [36] The tissues were typically fixed in 10% neutral buffered formalin immediately upon removal to prevent autolysis. The fixed tissue is dehydrated through increasing concentrations of alcohol (e.g., 70% to 100%), cleared in xylene, and embedded in paraffin wax. Using a microtome, the paraffin block is cut into thin sections (5 μm) by cross-section to display the horseshoe-shaped cartilage, the lumen, and the posterior trachealis muscle. The staining procedure using the Hematoxylin & Eosin (H&E) technique shows the general morphology (basophilic nuclei and eosinophilic connective tissue). The number of goblet cells and the thickness of the mucosal epithelium were observed using a light microscope at 400X magnification.

Lung tissue was removed through a thoracotomy procedure, then handled, processed, and stained with H&E as standard for histopathological examination. Using a light microscope at 400X magnification, the alveolar perimeter length, the degree of alveolar wall damage, and the number of inflammatory cells were measured in a blinded manner by two pathologists.

2.9.5. Histopathology Measurements

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 May 2026 doi:10.20944/preprints202605.1119.v1

The tracheal preparation was made from a transverse section of the trachea and analyzed with a light microscope at 400X magnification by an Anatomical Pathology Specialist. The number of goblet cells will be measured using the GCD (Goblet Cells Density) method. At the same time, the thickness of the mucosal epithelium will also be measured from selected 5 fields of view per tracheal ring. If the entire circumference is visible, researchers often count four specific quadrants (12, 3, 6, and 9 o'clock positions). We used quantitative methods, including Linear Density (Cells per mm), which measures the number of goblet cells along a specific length of the airway. Using imaging software (ImageJ®), the length of the epithelial basement membrane is measured in millimeters (L). The number of cells was the count of all positive goblet cells that touch the basement membrane (N); GCD = N (cell number)/L (length of the basement membrane in millimeters).

The measurement method for the thickness of the tracheal mucosal epithelium is linear measurement using a calibrated ocular micrometer or digital imaging software. Measurements are taken from the apical surface (excluding the cilia) down to the basement membrane. To ensure accuracy, 5 fields of view per transection of the tracheal ring were measured. Measurements are typically taken at multiple standardized points around the tracheal ring to calculate a mean value, which is recorded in micrometers (μm).

Under a microscope connected to Cellsens Olympus Imaging Software, the circumference of the alveolus was measured on 5 different viewpoints in 400x optical magnification by two pathologists with μm accuracy. The distribution of inflammatory cells was counted in 100X magnification, also in 5 different viewpoints, and subjectively classified into three categories: 1) Light distribution of inflammatory cells scored 1, 2) Medium distribution of inflammatory cells scored 2, and 3) Heavy distribution of inflammatory cells scored 3. The damage of alveolus lining cells viewed in 100x magnification on 5 different views which then categorized based how many damaged alveoli found on any given viewpoint as follows: 1) No damage scored 0, 2) Less than quarter of a total alveolus in each viewpoint scored 1, 3) More than a quarter yet less than a half of a total alveolus in each viewpoint scored 2, 4) More than half up to 75% of a total alveolus in each viewpoint scored 3, 5) Only less than 25% healthy alveoli found scored 4.

2.9.6. IL-6 and TNF-α Examination

Blood samples of 5 mL per rat were collected via the retro-orbital sinus into tubes containing EDTA. Samples should be centrifuged at approximately 1,000-2,000X g for 15 minutes at 4°C. Plasma must be separated immediately and assayed on the same day to avoid repeated freeze-thaw cycles, as these significantly reduce the detectable levels of IL-6 and TNF-α. Using Kit ELISA IL-6 (RAT) Elabscience E-EL-R0015 (96 well) and Kit ELISA TNF-α (RAT) Reedbio RE1060R (96 well). The mechanical steps of Sandwich ELISA procedures (Add sample -> Incubate -> Wash -> Add Detection -> Wash -> Add HRP -> Wash -> Color) for running those kits are identical. Ensure you use the specific Biotinylated Antibody and Standard provided in each respective box—they are not interchangeable between kits.

Before starting, ensure that all reagents and samples reach room temperature (18°C-25°C) and that the plasma is not hemolyzed, as released intracellular components can interfere with IL-6 and TNF-α measurements. detection. Dilute the 25X Wash Buffer concentrate with deionized water to create a 1x working solution. Reconstitute the Rat IL-6/TNF-α standard with the provided Reference Standard & Sample Diluent. Perform a 2-fold serial dilution to create a standard curve (e.g., 1000, 500, 250, 125, 62.5, 31.25, 15.63, and 0 pg/mL). Dilute the 100X concentrate to a 1X working solution using the Biotinylated Detection Ab Diluent (prepare only what is needed for the day). Dilute the 100x HRP Conjugate to 1x using the HRP Conjugate Diluent.

Add 100 μL of the prepared standards, blank, and plasma samples into the appropriate wells. Cover the plate with the sealer and incubate for 90 minutes at 37°C (during this phase, the IL-6 in the plasma binds to the capture antibody coated on the plate). Discard the liquid from each well. Immediately add 100 μL of the 1X Biotinylated Detection Ab working solution to each well. Incubate for 1 hour at 37°C. This antibody binds to a different epitope on the IL-6/TNF-α. molecule, forming

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 May 2026 doi:10.20944/preprints202605.1119.v1

the "sandwich". Aspirate the liquid from each well and add 350 µL of wash buffer (first wash). Let it soak for 1–2 minutes, then discard the liquid and pat the plate dry against clean absorbent paper. Repeat this process 3 times. Add 100 µL of 1X HRP Conjugate working solution to each well. Incubate for 30 minutes at 37°C (The Streptavidin-HRP binds to the biotin on the detection antibody). Aspirate and wash the wells 5 times (second wash) using the same process described in Step 3. This step is critical to remove any unbound HRP, which could cause high background noise. Add 90 µL of Substrate Reagent (TMB) to each well. Cover the plate and incubate for about 15 minutes at 37°C. Protect the plate from light. The solution will turn blue in wells containing IL-6/TNF-α. Add 50 µL of Stop Solution to each well. The color will immediately change from blue to yellow. Immediately measure the optical density (OD value) of each well using a microplate reader set to 450 nm. Using the ELISA Reader BioTek - ELx800 Absorbance Microplate Reader.

2.9.7. SOD-3 and MDA Examination

The core concept remains the same as in the detection of IL-6/TNF-α by Sandwich ELISA. Still, there are critical procedural differences between the Kit ELISA MDA (RAT) Abclonal RK15281 and the Kit ELISA SOD-3 (RAT) Reedbio RE2412R, which are used as reagents. The primary difference lies in the loading sequence. ABclonal often uses a "step-by-step" binding approach, while many modern kits (including some Reedbio lines) use a "simultaneous" incubation for the primary capture and detection.

Key operational differences in: 1) Incubation Times (The "Speed" Difference); ABclonal MDA is a longer assay, the total incubation time before color development is approximately 4 hours. Reedbio SOD-3 follows the same timing as your TNF-α kit, with a total incubation time of approximately 3 hours. 2) Reagent Preparation (HRP vs. Streptavidin-HRP); ABclonal often uses a Concentrated Streptavidin-HRP that requires a specific 1:100 dilution in a dedicated "HRP Diluent". Reedbio: Usually provides a Concentrated HRP Conjugate, 3) Standard Curves & Units; MDA is usually measured in ng/mL or nmol/mL. SOD-3 is usually measured in ng/mL or pg/mL.

2.10. Ethical Statement

These procedures were approved by the Health Research Ethics Commission of the Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia, with protocol number 088/EC-H/KEPK/FK-UNDIP/VIII/2024. No generative artificial intelligence (GenAI) is used in this paper.

3. Results

For each experiment conducted, including independent replications, this study compared the 8 and 12-week exposure times and the effects of nicotine and AA in vaping versus cigarette smoke. The measures of variability in body weight, plasma levels of IL-6, TNF-α, SOD3, and MDA, and the pathological changes in the trachea-alveolar region were described and analyzed.

Figure 1 shows the total enrollment of the 30 subjects, allocation, follow-up, and, finally, the analysis showing no dropouts or exclusions.

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 May 2026 doi:10.20944/preprints202605.1119.v1

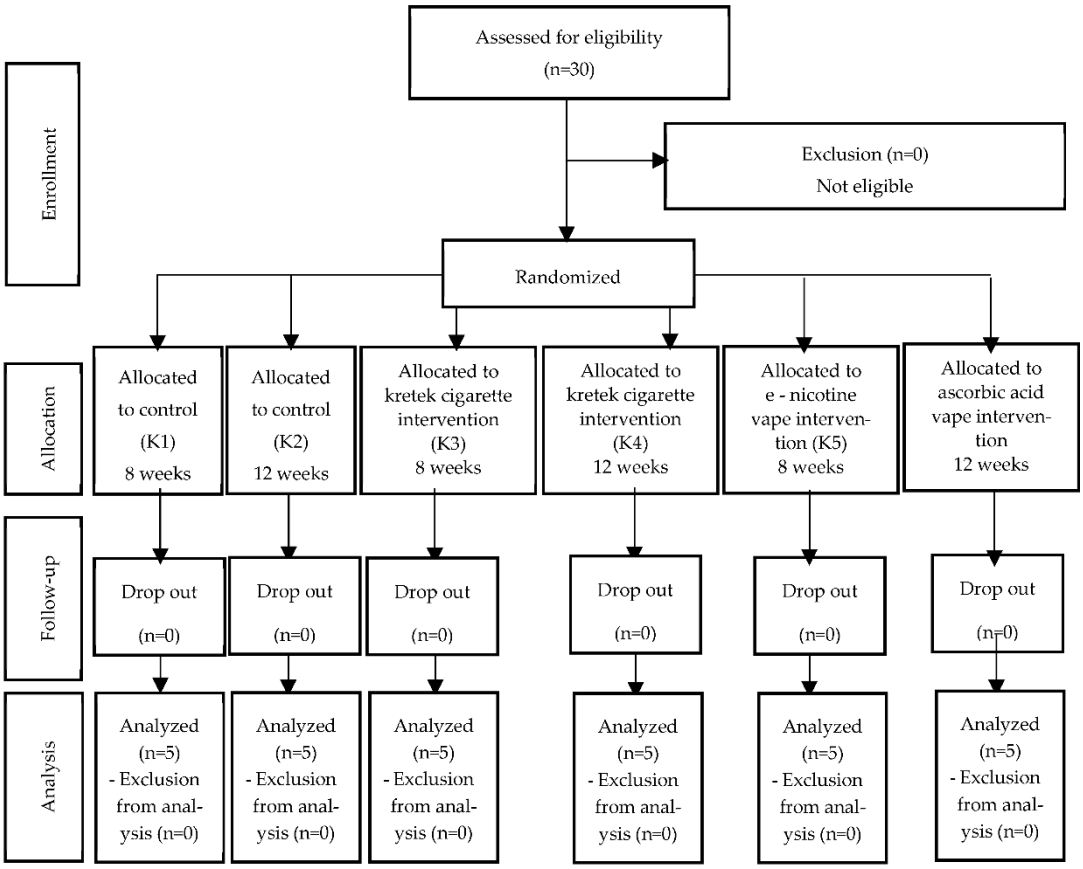

Figure 1. The Consort Diagram.

Table 1 shows body weight changes before and after the tests for groups K1-K6. K1 had high variation after the test (SD = 44.64), with an overall SD of 44.75. K2 had stable weights before (SD = 2.30) but greater variation after (SD = 37.80), resulting in a combined SD of 37.87. K3 increased by an average of 141.0 grams, but the SD rose from 4.66 to 32.10, indicating greater variability among individuals. K4 had an average increase of 32.8 grams and a combined SD of 36.62, showing wide variation. K5 showed a slight average increase and a combined SD of 21.77, close to the average difference of 25.6 grams. K6 had a significant average increase of 68.6 grams and a combined SD of 21.79, indicating consistent changes despite more variability. These data suggest that short-term cigarette exposure (K3) did not hinder weight gain. In contrast, electronic nicotine delivery (K5) and long-term cigarette use (K4) reduced expected weight gain compared with the control groups.

Table 1. The mean ± SD of body weight differences pre- and post-tests between groups.

Groups	**Pre-test**	**Post-test**	(Δ) Mean±SD	Sig.	Tukey's
	Mean±SD (g)	Mean±SD (g)	(g)	p	Post-Hoc
K1 (n=5)	168.8 ± 3.11	307.4 ± 44.64	138.6 ± 44.75	<0.001	a
K2 (n=5)	165.4 ± 2.30	302.8 ± 37.80	137.4 ± 37.87	<0.001	a
K3 (n=5)	165.8 ± 4.66	306.8 ± 32.10	141.0 ± 32.44	<0.001	a
K4 (n=5)	179.8 ± 13.55	212.6 ± 34.02	32.8 ± 36.62	<0.001	b
K5 (n=5)	169.6 ± 10.06	195.2 ± 19.31	25.6 ± 21.77	<0.001	b
K6 (n=5)	171.8 ± 6.69	240.4 ± 20.74	68.6 ± 21.79	<0.001	c

Notes: K1 = control 8 week, K2 = control 12 week, K3 = cigarette 8 week, K4 = cigarette 12 week, K5 = e-nicotine 8 weeks, K6 = e-ascorbic acid 12 weeks. One-Way ANOVA, $F(5, 24) = 10.22$, $p < 0.001$. Tukey's Post-Hoc Test ($\alpha = 0.05$); a = significantly different from K4 and K5; b = significantly different from K1, K2, and K3; c = significantly different from K4 and K5, but not significantly different from K1, K2, or K3.

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 May 2026 doi:10.20944/preprints202605.1119.v1

There is a big difference between the highest weight gain (141.0g) and the lowest (25.6g). This will likely show a significant result with One-Way ANOVA ($p < 0.05$), requiring a Post-Hoc test. Tukey's test compares every pair of groups. The groups form three main "statistical clusters." In Cluster A (normal growth: Groups K1, K2, K3), the weight difference between the control group (K1 at 138.6g) and the group that smoked three cigarettes for 8 weeks (K3 at 141.0g) is only 2.4g. This difference is not significant ($p > 0.05$), indicating that smoking three cigarettes daily for 8 weeks does not significantly affect growth. Cluster B shows significant growth inhibition in the K4 and K5 groups. Group K5, using E-nicotine vapes for 8 weeks, had an average weight of 25.6g, while Group K4, smoking cigarettes for 12 weeks, weighed 32.8g. Both groups show a significant weight difference compared to the control group ($p < 0.05$) but are similar to each other. Cluster C shows moderate growth inhibition in Group K6, which used E-ascorbic acid vapes for 12 weeks, with an average weight of 68.6g. This group also has a significant weight difference compared to the control ($p < 0.05$) and the E-nicotine group (K5). This suggests that the ascorbic acid vape affects growth less than the nicotine vape and long-term smoking.

Table 2 shows how cigarette smoke, e-nicotine, and e-ascorbic acid vapor affect IL-6 and TNF-α. At 12 weeks, the cigarette group (K4) and the e-ascorbic acid group (K6) had the highest IL-6 levels, 11.45 ± 1.17 and 11.83 ± 1.56, respectively. Both groups had higher IL-6 levels than the 8-week group and the 12-week control group. There was no significant difference in IL-6 levels between K4 and K6 at 12 weeks. Overall, IL-6 levels varied significantly across all groups ($F(5, 24) = 56.6$, $p < 0.001$). Significant differences were observed for TNF-α ($F(5, 24) = 81.1$, $p < 0.001$). High-response groups may be observed in the 8-week groups K1, K3, and K5, which showed significantly higher TNF-α levels than all 12-week groups. Low-response groups were identified at the 12-week groups (K2, K4, K6) that showed a marked decrease in TNF-α concentrations compared to the 8-week cohorts.

Table 2. The Comparison of IL-6, TNF-α, SOD3, MDA Plasma Levels Between Groups.

Groups	Variables (Mean±SD)			
	IL-6 [a]	TNF-α [b]	SOD3 [c]	MDA [d]
K1 (n=5)	5.73 ± 0.87	77.2 ± 9.98	11.85 ± 1.03	154.30 ± 64.24
K2 (n=5)	8.43 ± 0.88	18.76 ± 2.48	10.06 ± 0.77	219.20 ± 83.04
K3 (n=5)	6.4 ± 0.69	76.9 ± 9.44	12.75 ± 1.10	198.47 ± 119.30
K4 (n=5)	11.45 ± 1.17	21.97 ± 4.33	9.33 ± 0.10	243.15 ± 106.00
K5 (n=5)	6.5 ± 1.14	66.1 ± 9.80	11.05 ± 2.81	243.89 ± 242.66
K6 (n=5)	11.83 ± 1.56	18.19 ± 1.62	9.07 ± 0.13	178.23 ± 53.30

Notes: K1 = control 8 week, K2 = control 12 week, K3 = cigarette 8 week, K4 = cigarette 12 week, K5 = e-nicotine 8 weeks, K6 = e-ascorbic acid 12 weeks. One-Way ANOVA: IL-6: $F(5, 24) = 56.6$, $p < 0.001$; TNF-α: $F(5, 24) = 81.1$, $p < 0.001$; SOD3: $F(5, 24) = 10.08$, $p < 0.001$; MDA: $F(5, 24) = 0.447$, $p = 0.812$ (not significant); Tukey's Post-Hoc Test ($\alpha = 0.05$).

(a) IL-6 : K4 and K6 significantly higher than K1, K2, K3, and K5 ($p < 0.05$). No significant difference
(b) K4 and K6 ($p > 0.05$).
(c) TNF-α: K1, K3, and K5 significantly higher than K2, K4, and K6 ($p < 0.05$).
(d) SOD3 : K3 significantly higher than K2, K4, and K6 ($p < 0.05$). K1 significantly higher than K4 and K6 ($p < 0.05$).
(e) MDA: No significant pairwise differences ($p > 0.05$ for all comparisons).

Table 2 also presents the SOD3 levels, which varied significantly across the study ($F(5, 24) = 10.08$, $p < 0.001$). The highest antioxidant activity was in group K3 (12.75 ± 1.10), which was significantly higher than all 12-week groups (K2, K4, K6). The 8-week control (K1) also maintained significantly higher SOD3 levels than the 12-week cigarette (K4) and e-ascorbic acid (K6) groups. Unlike the other markers, MDA levels showed no significant variation among the groups ($F(5, 24) =$

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 May 2026 doi:10.20944/preprints202605.1119.v1

0.447, p = 0.812), and Tukey's test confirmed no significant pairwise differences in lipid peroxidation (MDA) across interventions or durations.

Based on the data provided in Table 3, the following is a descriptive analysis of the tracheal and alveo-pulmonary histopathology results across the six study groups. The density of goblet cells showed a statistically significant variation across groups (F (5, 24) = 3.235, p = 0.022). The group K3 exhibited the highest density (4.2 ± 2.44 N/mm), followed by K4 (3.93 ± 2.67 N/mm). Despite the significant ANOVA, Tukey's HSD test revealed no specific significant pairwise differences. The difference between K3 and the K1 control was noted as borderline but did not reach the significance threshold (difference of 3.07 vs. the required 3.227).

Mucosal thickness varied significantly among the groups (F(5, 24) = 9.40, p < 0.001). The peak measurements were at the 8-week intervention groups, K5 (e-nicotine) and K3 (Cigarette), which recorded the thickest mucosa at 71.03 ± 13.59 μ and 66.88 ± 17.92 μ, respectively. Mucosal thickness in K3 and K5 was significantly greater than in the 12-week groups, including K2 (Control), K4 (Cigarette), and K6 (e-ascorbic acid).

Table 3. The comparison of tracheo-alveo-pulmonary histopathological between groups.

Groups	Tracheal Histopathology		Alveo-pulmonary Histopathology		
	Goblet Cells Density[a] Mean±SD (N/mm)	Mucosal Thickness[b] Mean±SD (μ)	Alveolar circumference[c] Mean±SD (μ)	Total Score of the distribution of inflammatory cells	Total Score damage of the alveolus lining cells
K1 (n=5)	1.13 ± 0.52	52.40 ± 2.63	202.03 ± 29.38	6	6
K2 (n=5)	1.37 ± 0.72	45.50 ± 7.48	273.58 ± 45.28	7	7
K3 (n=5)	4.2 ± 2.44	66.88 ± 17.92	469.77 ± 91.31	5	5
K4 (n=5)	3.93 ± 2.67	34.65 ± 6.55	221.17 ± 47.64	6	6
K5 (n=5)	1.73 ± 1.05	71.03 ± 13.59	362.99 ± 51.2	6	6
K6 (n=5)	2.89 ±1.18	42.10 ± 6.54	261.61 ± 58.56	7	7
Variabel					
F (df)	F (5,24) = 3.235	F (5,24) = 9.40	F (5,24) = 16.72		
p-value	p = 0.022*	p < 0.001	p < 0.001		
Tukey's HSD (α=0.05)	No significant pairwise differences (K3 vs K1 borderline)	K3, K5 > K4, K2, K6	K3 > K1, K2, K4, K6; K5 > K1, K4		

Notes: K1 = control 8 week, K2 = control 12 week, K3 = cigarette 8 week, K4 = cigarette 12 week, K5 = e-nicotine 8 weeks, K6 = e-ascorbic acid 12 weeks.

One-Way ANOVA

(a) Goblet Cells Density (N/mm), F = 3.235, *p = 0.022 (p < 0.05, significant)
(b) Mucosal Thickness (μ), F = 9.40, p < 0.001 (F critical 2.62)
(c) Alveolar Circumference, F (5, 24) = 16.72, p < 0.001.

Tukey's Post-Hoc Test

(a) Goblet Cells Density = Significant if the difference in mean > 3.227.

- K3 vs K1 (4.20 - 1.13 = 3.07) → ✗ (No significant difference, although it's close)
- K3 vs K2 (2.83) ✗; K3 vs K5 (2.47) ✗; K4 vs K1 (2.80) ✗; K4 vs K5 (2.20) ✗; K6 vs K1 (1.76) ✗

(b) Mucosal Thickness = Significant if the difference in mean > 20.41

- K3, K5 > K4, K2, K6
- K5 vs K1 (18.63) ✗; K3 vs K1 (14.48) ✗

(c) Alveolar Circumference = Significant if the difference in mean > 20.41

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 May 2026 doi:10.20944/preprints202605.1119.v1

K3 significantly higher than K1, K2, K4, and K6 ($p < 0.05$), K5 significantly higher than K1 and K4 ($p < 0.05$), K3 vs K5: $p = 0.051$ (not significant, but borderline). No other significant differences ($p > 0.05$)

The qualitative scoring of total scores for inflammatory cell distribution and alveolar lining cell damage Table 3) showed that inflammatory cell distribution in the 12-week control (K2) and the 12-week e-ascorbic acid group (K6) both received the highest score of 7. In contrast, the 8-week cigarette group (K3) received the lowest score of 5. Alveolar lining cell damage was similar to the inflammation scores, with K2 and K6 showing the highest damage scores (7), while K3 showed the lowest (5).

Figure 2 showed that in cigarette exposure (Figure 2C and 2D), goblet cell density decreased, followed by squamous metaplasia, in which the flexible ciliated epithelium is replaced by tougher, non-functional cells. The alveolar walls begin to thin and rupture (early emphysema). The lowest goblet cell density was observed at E-nicotine exposure for 8 weeks (C) and generally shows milder irritation, though high concentrations of propylene glycol can cause early dehydration of the epithelial surface, resulting in E-ascorbic acid for 12 weeks (F).

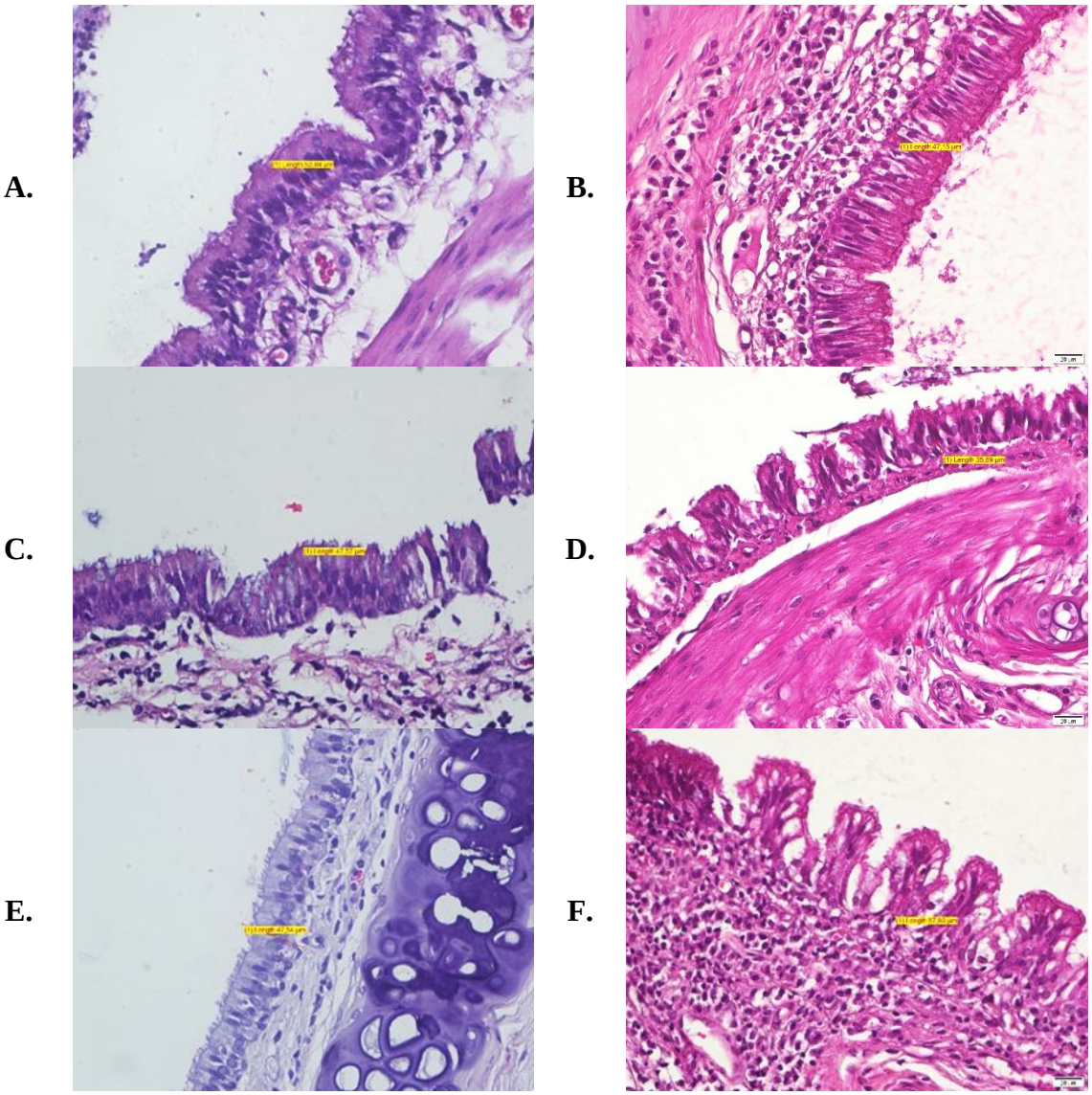

Figure 2. The comparative-histopathology of tracheal transection between groups. Tracheal Histopathology under light microscope in 400x magnification; A. K1 = control 8 weeks, B. K2 = control 12 weeks, C. K3 = cigarette 8 weeks, D. K4 = cigarette 12 weeks, E. K5 = e-nicotine 8 weeks, and F. K6 = e-ascorbic acid 12 weeks.

Figure 3 pointed that the alveolar walls begin to thin and rupture (early emphysema) in Figure 3C, 3D and 3E. The alveolar circumference of K3 (Figure 3C) > K1 (Figure 3A), K2 (Figure 3B), K4 (Figure 3E), and K6 (Figure 3F). In phytochemical intervention, that e-liquid contains protective

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 May 2026 doi:10.20944/preprints202605.1119.v1

phytochemicals (ascorbic acid), studies often show a preservation of the basement membrane and a significant reduction in the thickening of the alveolar septa (Figure 3F) compared to nicotine-only groups (Figure 3E).

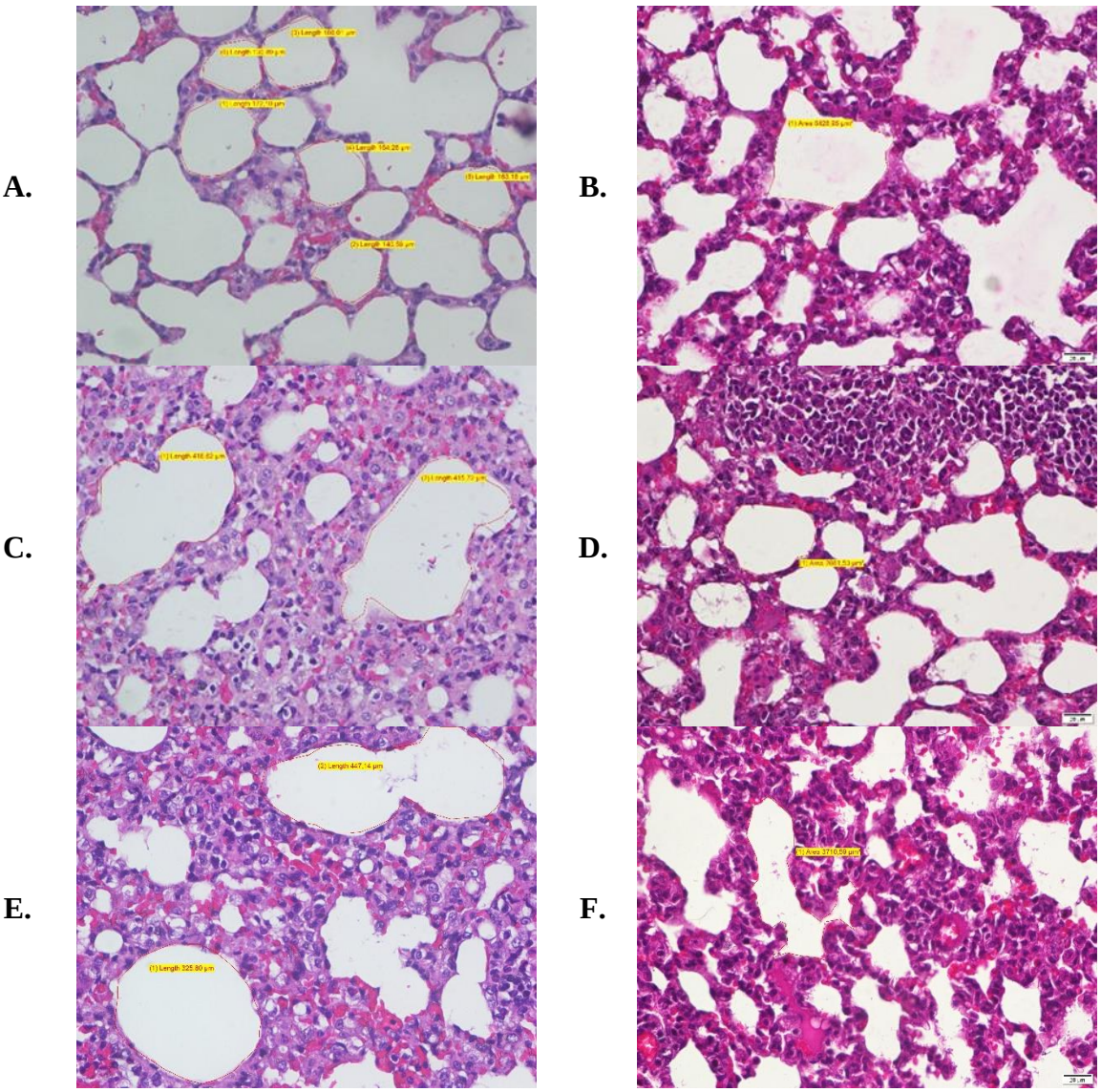

Figure 3. The Comparative-Histopathology of Alveo-pulmonary Between Groups. Alveo-pulmonary under light microscope in 400x magnification; A. K1 = control 8 weeks, B. K2 = control 12 weeks, C. K3 – cigarette 8 weeks, D. K4 = cigarette 12 weeks, E. K5 = e-nicotine 8 weeks, and F. K6 = e-ascorbic acid 12 weeks.

4. Discussion

4.1. Body Weight Change

The relationship between the weight-suppressing effect of smoking is more complex, particularly regarding "metabolic efficiency" and long-term health. While smokers often have a lower overall BMI, Yu et al. (2018) highlight that they frequently have a higher waist-to-hip ratio (WHR). This suggests that smoking may promote "unhealthy" metabolic efficiency by redistributing fat to the abdominal area, which increases the risk of insulin resistance and metabolic syndrome despite a lower total weight [37]. Research also suggests that while acute nicotine exposure increases metabolic rate, chronic exposure may eventually lead to a decrease in metabolic rate and an increase in appetite over very long durations, as the body adapts or as tobacco-related illnesses (like COPD) limit physical activity [38].

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 May 2026 doi:10.20944/preprints202605.1119.v1

Smoking three cigarettes daily for 12 weeks leads to oxidative stress and reduced energy use, resulting in less weight gain compared to a control group and an 8-week exposure group. The larger weight difference in the 8-week group is likely due to body adaptation or immediate damage. While weight gain is typical with age, those exposed to smoke gain significantly less weight ($p < 0.05$) than the control group. Research by Chen et al. (2008) shows weight gain is stable in the first 8 weeks but decreases further by 12 weeks. Smoke-exposed individuals are lighter than the control and "pair-fed" groups, indicating that weight loss results from both reduced food intake and metabolic changes [39].

Cigarette smoke affects the body significantly around week 8, causing acute damage and high levels of inflammation and oxidative stress. By week 12, the body starts to adapt, reducing these responses. The smoke contains ROS, which disrupts the body's balance, leading to higher damage markers such as MDA and lower levels of natural antioxidants such as glutathione. Cigarette smoke raises the levels of Uncoupling Proteins (UCPs), especially UCP1 in brown fat and UCP3 in muscle. These proteins convert energy into heat rather than storing it, wasting calories and reducing energy efficiency [39].

Research indicates that body weight changes depend on two factors: the type of substance used (e-nicotine or e-ascorbic acid) and the exposure duration (8 weeks or 12 weeks for traditional cigarettes). The most significant differences in weight gain occur when comparing any control group to e-nicotine users or long-term smokers. The findings suggest serious damage from cigarette smoke and e-liquid aerosols over time. E-nicotine rapidly increases TNF-α levels, leading to growth inhibition and tissue irritation. Long-term smoking causes ongoing increases in IL-6 and TNF-α, resulting in tissue damage and weight loss. Conversely, vaping e-ascorbic acid reduces inflammation and supports better weight retention compared to nicotine. In conclusion, body weight differentiation is a visible result of the internal struggle between oxidative stress (from smoke/nicotine) and antioxidant defense (from ascorbic acid), mediated by the IL-6/TNF-α inflammatory axis in the respiratory system.

4.2. The Comparative Effects on IL-6 and TNF-α Levels

The data show significant variation for IL-6, TNF-α, and SOD3, but not for MDA. This discrepancy raises questions about which pathways are primarily targeted by these specific phytochemical aerosols versus those targeted by traditional cigarette smoke. A significant pro-inflammatory response: long-term exposure (12 weeks) to both cigarette smoke and e-ascorbic acid vapor results in significantly higher IL-6 levels compared to 8-week cohorts and controls. This supports the consensus that chronic inhalation of aerosols, regardless of the base, can trigger systemic inflammation [40]. The 8-week exposure with moderate elevation reflects acute compensation, with the body managing the irritation. The 12-week exposure with significant spike levels indicates a transition to chronic systemic inflammation, which increases the risk of cardiovascular and metabolic diseases.

By showing that e-ascorbic acid vapor, often perceived as a “healthier” or antioxidant-rich alternative, elicits an IL-6 response similar to that of cigarette smoke, the study highlights that the physical state of the substance (aerosol/vapor) may be as problematic as its chemical composition. Surprisingly, the e-ascorbic acid group (K6) showed IL-6 levels (11.83 ± 1.56) comparable to the cigarette smoke group (K4) (11.45 ± 1.17). In many respiratory studies, phytochemicals are expected to be protective; these results suggest that the aerosolization process or concentration of ascorbic acid might negate its typical antioxidant benefits, potentially even acting as a pro-oxidant under specific conditions [41,42].

High levels of IL-6 are a gold-standard marker for systemic inflammation. Finding consistent elevations across different aerosol bases supports the “General Irritant Theory”, which suggests that the lungs' immune response can be triggered by repeated inhalation of fine particles, regardless of their composition including the base components of e-liquids propylene glycol (PG) and vegetable glycerin (VG) can induce oxidative stress and inflammatory responses in the lungs, independent of nicotine or tobacco [43,44,45].

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 May 2026 doi:10.20944/preprints202605.1119.v1

When both a known toxin, like cigarette smoke, and a neutral substance, such as ascorbic acid, produce the same inflammatory marker, it becomes harder to pinpoint the toxicological pathways [46]. The IL-6 alone doesn't tell us the full story of organ-specific damage. For example, cigarette smoke might cause DNA damage or cancer that e-ascorbic acid does not, even if their IL-6 levels are temporarily similar. But smoking status and inflammatory markers confirm that IL-6 is a primary driver of chronic elevation in C-reactive protein (CRP) and is significantly higher in long-term smoke-exposed subjects compared to controls [47]. Nevertheless, ascorbic acid is typically an anti-inflammatory, and this result suggests that when delivered via heat/vaporization, its chemical properties might change (degrade) or its physical presence in the alveoli might override its antioxidant benefits, which could be misleading to consumers [48,49].

A common phenomenon in immunology, the shifting “wave” of inflammation, was studied in this research. It suggests that as time passes, the body moves from a high-energy, acute response to a more sustained, chronic state. Time-dependent cytokine dynamics were reflected in the observation that TNF-α levels were significantly higher in the 8-week groups than in the 12-week groups, suggesting a complex, time-dependent immune response. This may reflect an acute-to-chronic transition in the subjects' inflammatory profile.

It is well-documented that TNF-α is a “first responder”. High levels at 8 weeks followed by a decline at 12 weeks align perfectly with the transition from an acute inflammatory phase to a regulatory or chronic phase [50,51]. Instead of a single “snapshot”, comparing two time points (8 vs. 12 weeks) provides a “movie” of the immune system. This allows researchers to see the resolution phase of inflammation. A drop in TNF-α doesn't always mean the inflammation is “resolving”. The immune system might simply be switching to different cytokines (like IL-6 or IL-10). Using only TNF-α to define a “transition” can be misleading [52].

Identifying the peak TNF-α level (8 weeks) and subsequent drop (12 weeks) helps clinicians understand the "window of opportunity" for anti-inflammatory treatments. Lower levels at 12 weeks could indicate immune exhaustion rather than a healthy transition. If the stimulus (infection/injury) is still present, the body might just be unable to keep up production.

The standard inflammatory timeline reminds us that the acute phase (weeks 1–8) is characterized by high concentrations of pro-inflammatory cytokines (TNF-α, IL-1β) that recruit immune cells to the site of injury. The transition phase (weeks 8–12) explained that the body attempts to reach homeostasis, and pro-inflammatory markers begin to dip. Finally, the chronic phase (week 12+) pointed out that if the issue isn't resolved, the profile shifts toward tissue remodelling or persistent low-grade inflammation [53,54].

4.3. The Comparative Effects on SOD3 and MDA Levels

The study highlights a significant reduction in SOD3 (antioxidant depletion) in 12-weeks exposure groups compared to 8-week groups. This aligns with recent literature suggesting that prolonged exposure to smoke or vapor induces oxidative stress that overwhelms the body’s primary antioxidant defenses [55]. Unlike the significant changes observed in SOD3, MDA levels (a marker of lipid peroxidation) showed no significant variation across groups ($p = 0.812$). This lack of significance is unusual in models of chronic smoke exposure, where MDA typically rises as SOD3 falls. This could suggest that the 12-week duration was insufficient to cause measurable permanent lipid damage or that the sample size lacked the power to detect these differences.

The primary evidence of harm in both the cigarette and e-liquid groups is the significant reduction in SOD3 levels over time. SOD3 is a critical extracellular enzyme that neutralizes superoxide radicals, protecting tissues from oxidative damage [56]. In the study, long-term suppression was observed in the 12-week groups (K4: Cigarette and K6: e-ascorbic acid), which showed the lowest SOD3 levels (9.33 ± 0.10 and 9.07 ± 0.13, respectively), significantly lower than those of the 8-week control group (K1). This implies significantly decreased activity compared to controls, indicating antioxidant exhaustion.

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 May 2026 doi:10.20944/preprints202605.1119.v1

Cigarette smoke exposure in an 8-week period indicates higher antioxidant activity by SOD-3 enzyme, which aligns with a previous study by Tang et al. (2022) that stated there's an enhancement of antioxidant activity in increased oxidative stress level as a protective response in 2-week exposure to heat stress [57]. Meanwhile, if the exposure period is prolonged, the antioxidant activity of the SOD-3 enzyme will be reduced due to harmful ingredients in smoking devices, including vapes and cigarettes, which cause dysregulation of antioxidant enzyme production. A lower SOD level was found in chronic smokers compared to novice smokers, attributable to a disrupted IL-6-mediated SOD pathway and increasing cotinine levels [58]. It has been proven that the SOD level in the heavy-smoking group is lower than that of the control non-smoking group, stressing the effect of cigarette consumption on antioxidant activity and oxidative stress levels in the body [59]. Exposure to ascorbic acid-base vape aerosol over 12 weeks indicates lower antioxidant activity by SOD-3 enzyme, likely affected by the impaired antioxidant enzyme expression caused by chronic inflammation. The ascorbic acid-based vape aerosol does not affect the expression of SOD-3 enzyme. In their publication, Bezerra et al. (2023) stated that increased oxidative stress levels caused by inflammatory cells in asthmatic patients ultimately impaired SOD activity [60] Other components in the aerosol, such as glycerin, propylene glycol, and flavoring, play a role in the upper airway pathway [61].

The SOD-3 levels between 8-week and 12-week exposures show no significant difference ($p>0.05$). It can be interpreted that different exposure periods do not affect the SOD-3 enzyme expression, whether in the control group, the cigarette-exposed group, or the vape aerosol-exposed group with different e-liquid bases.

Even "phytochemical-based" aerosols like e-ascorbic acid (K6) did not prevent this decline, suggesting that the delivery method (aerosolization) or the substances themselves may still provoke a strong oxidative response that exhausts the body's natural antioxidant pool. While the study found no statistically significant differences in MDA levels across groups ($p = 0.812$), cigarette smoke is academically established as a catalyst for lipid peroxidation. General research indicates that acrolein and other particulates in smoke/aerosols trigger ROS that attack cell membranes, leading to increased MDA [62]. The lack of significance in this specific study (MDA: $F(5, 24) = 0.447$) may be due to the short 12-week timeframe or the limited sample size, rather than a lack of biological effect.

Nicotine-based vape aerosol exposure over an 8-week period shows the highest oxidative stress, as indicated by MDA levels, suggesting that nicotine in vape aerosol induces lipid peroxidation and increases MDA production. Followed by the group with cigarette smoke exposure in 12 weeks, further proving that nicotine prominently contributes to the increasing MDA level in the body, which indicates higher oxidative stress. Ascorbic acid-base vape aerosol exposure in a 12-week period has the lowest MDA level, which can be interpreted as indicating that this treatment caused a lesser oxidative burden, potentially caused by the ascorbic acid contained in the vape aerosol acting as a free-radical scavenger. Ascorbic acid has the ability to stabilize free radicals by donating electrons directly without a metabolic process, resulting in protection and repair caused by oxidative damages [63,64]. The MDA levels between 8-week and 12-week exposures show no significant difference ($p > 0.05$). It can be seen that different exposure periods do not significantly affect MDA production in the control group, the cigarette-exposed group, or the vape aerosol-exposed group across different e-liquid bases.

The "hormetic" effect found in this research suggests that the body may initially mount an adaptive defense against smoke [65]. At the 8-week peak, the highest SOD3 activity was observed in the 8-week cigarette group (K3: 12.75 ± 1.10), which was significantly higher than in all 12-week groups. This aligns with the "oxidative eustress" theory, where low-to-moderate levels of ROS initially upregulate antioxidant enzymes as a protective measure before the system becomes overwhelmed [66]. Despite exposure to nicotine and aerosols, there were no significant pairwise differences in lipid peroxidation at any time point or across interventions. This suggests that within a 12-week period, the body's regulatory systems may still be maintaining membrane integrity despite the decline in SOD3 [67].

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 May 2026 doi:10.20944/preprints202605.1119.v1

While K6 (e-ascorbic acid) had low SOD3 at 12 weeks, the use of phytochemicals in e-liquids is often explored to mitigate smoke-induced damage. The potential benefit, based on the literature, is that exogenous antioxidants, such as ascorbic acid, can decrease lipid peroxidation following smoke exposure. However, the study results suggest that when delivered as an e-liquid aerosol, this protective effect might be negated by an inflammatory response, as evidenced by the high IL-6 levels in K6 [68].

The study shows that both cigarette smoke and e-ascorbic acid aerosols significantly deplete antioxidant defenses (SOD3) and increase inflammation over 12 weeks. The body may attempt an early defense (seen at 8 weeks), and systemic lipid damage (MDA) may take more than 12 weeks to become significant.

4.4. Temporal Progression of Tracheo-Alveolar Histopathology

The respiratory system responds to inhaled insults through a sequence of structural adaptations and eventual failures. The "temporal" aspect refers to how these tissues degrade over days, weeks, or months of exposure. Acute irritation occurs on days 1–7 (Phase 1) and can be triggered by cigarette smoke, which may cause a rapid onset of mucus hypersecretion and loss of ciliary beat frequency, and is accompanied by early neutrophil infiltration of the tracheal lining. Besides E-Liquid Aerosols exposure, it generally shows milder irritation, though high concentrations of propylene glycol can cause early dehydration of the epithelial surfaces [69].

In the 4- to 12-week exposure period, the phase 2 cellular response, including chronic remodelling. Tracheal changes occur with cigarette smoke exposure, leading to squamous metaplasia, in which the flexible, ciliated epithelium is replaced by tougher, nonfunctional cells. Besides the alveolar destruction shown in the deep lung, where the alveolar walls begin to thin and rupture (early emphysema). If the e-liquid contains protective phytochemicals, studies often show preservation of the basement membrane and a significant reduction in alveolar septal thickening compared with nicotine-only groups [70].

This study reported significant microstructural changes in tracheal-alveolar tissue. In the shorter exposure (8 weeks) to cigarette smoke, the goblet cell density is denser than in 12 weeks. It means that the peak of acute exposure, the point of no return, occurred during the 8-week period, followed by irreversible injury that may decrease goblet cell density. The histopathology of the tracheal mucosal thickness also reflected this phenomenon. Unfortunately, this study cannot be supported by differences in the distribution of inflammatory cells or in damage to alveolar lining cells.

Cigarette smoke exposure in an 8-week period has the highest goblet cell counts, altering the upper airway structure composition, marked by the higher number of goblet cells in smokers compared to ex-smokers and never-smokers. Goblet cell counts change, accompanied by changes in mucin expression, suggesting that mucin production is likely influenced by goblet cell densities [71]. Besides goblet cells hyperplasia, cigarette smoke exposure also caused metaplasia in goblet cells, both of which eventually lead to hypersecretion of mucus, which causes respiratory problems [72,73]. The 8-week control group shows the lowest goblet cell counts, indicating that exposure to irritants would eventually affect tracheal structural composition, including goblet cell counts. The non-smokers group has lower goblet cell counts and higher ciliated cell counts than the smoker group, accompanied by better pulmonary functions [74]. The GCD between 8-week and 12-week exposures shows no significant difference ($p > 0.05$). It can be seen that different exposure periods do not significantly affect Goblet Cell Counts in the control, cigarette-exposed, or vape aerosol-exposed groups across e-liquid bases.

Exposure to nicotine-based vape aerosol over an 8-week period results in the thickest mucosal epithelial layer, consistent with a previous study indicating that vape aerosol composition is an irritant to the respiratory system and eventually causes mucus overproduction, resulting in a thickened mucosal epithelial layer [75]. Normally, when the trachea is inflamed and under oxidative stress, it results in thickening of the mucosal epithelium, but in some cases, chronic exposure to irritants can cause a reduction in mucosal epithelium thickness. Cigarette smoke exposure over 12

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 May 2026 doi:10.20944/preprints202605.1119.v1

weeks results in the thinnest mucosal epithelial thickness, consistent with a previous study indicating that cigarette smoke and vape aerosol accumulation could promote cell death, disrupt the protective barrier, and alter epithelial structure [76]. Exposure to cigarette smoke over a longer period alters the mucosal epithelium to a cuboidal structure with shortened, decreased cilia, reducing mucosal epithelial thickness in the trachea [77]. The Mucosal Epithelium Thickness between 8-week exposure and 12-week exposure shows a significant difference ($p<0.05$). It can be seen that different exposure periods significantly affect the thickness of the Mucosal Epithelial Layer. The Bonferroni Post Hoc test shows there's a significant effect of different period exposure in the cigarette smoke-exposed group and the vape aerosol-exposed group. The duration of exposure to irritants, including cigarette smoke and vape aerosol, could significantly affect tracheal mucosal epithelial thickness. Acute exposure to irritants usually causes the mucosal epithelium layer of the upper respiratory tract to thicken as a consequence of mucus hypersecretion [78]. Significant alteration of the structure of the mucosal epithelium layer in the trachea, and so the previous study proved that the duration of smoke exposure is positively correlated with the degree of tracheal pathological abnormality, marked by collapsed and damaged goblet cells, altered mucosal structure, and loss of cilia [79]. Alterations in the tracheal mucosal epithelium could reduce the effectiveness of respiratory tract defenses, making it easier for pathogens to enter the body through the airways.

4.5. The IL-6/TNF-α Molecular Signaling Axis

The IL-6 and TNF-α axis is the "alarm system" of the mucosal airways. When these proteins are elevated, they drive a feedback loop of chronic inflammation. Exposure to cigarette smoke triggers the NF-κB pathway, which acts as a master switch for 1) TNF-α, which is released by alveolar macrophages. It acts as a primary "recruiter" for other immune cells and induces cell death (apoptosis) in lung tissue. 2) IL-6 orchestrates the transition from acute to chronic inflammation. High temporal levels of IL-6 are strongly correlated with the severity of tissue scarring (fibrosis).

The data show a distinct pattern in how IL-6 and TNF-α respond to long-term exposure. Interleukin-6 levels are significantly higher in the K4 (cigarette 12 weeks) and K6 (e-ascorbic acid 12 weeks) groups compared to the controls and 8-week groups. This suggests that 12 weeks of exposure triggers a more robust chronic inflammatory signal than 8 weeks. Interestingly, TNF-α levels follow a different temporal pattern, with the 8-week groups (K1, K3, K5) significantly higher than the 12-week groups (K2, K4, K6). This may indicate a transition from an acute inflammatory phase (TNF-α dominant) to a chronic phase (IL-6 dominant) as the duration of exposure increases

The activation of the inflammatory axis often leads to systemic metabolic changes, as reflected in the weight-gain data. Groups with elevated inflammatory markers (specifically K4, K5, and K6) show significantly lower weight gain (Δ Mean) than the controls (K1, K2). While the controls (K1, K2) gained approximately 137g–138g, the 12-week cigarette group (K4) only gained 32.8g. This suppression of weight gain correlates with the peak IL-6 levels seen in Table 2, suggesting that chronic inflammation may be inhibiting normal growth or causing metabolic cachexia.

The signaling axis also correlates with the depletion of the body's antioxidant capacity. As IL-6 levels rise in the 12-week groups (K4 and K6), Superoxide Dismutase 3 (SOD3) levels significantly drop compared to the 8-week cigarette group (K3). The reduction in SOD3 at K4 and K6, alongside elevated IL-6, suggests that the inflammatory axis contributes to an environment in which oxidative stress is not effectively neutralized, potentially leading to further cellular damage.

4.6. Comparative Impact of Phytochemical-Based Aerosols

The data reveal a stark divergence in the biological trajectories of chronic cigarette smoke exposure and the introduction of phytochemical-based aerosols. This comparison highlights the potential of specific plant-derived compounds to modulate the IL-6/TNF-α signaling axis and preserve cellular architecture. Under the influence of traditional cigarette smoke, the molecular signaling axis exhibits a relentless linear increase. This upward trend in IL-6 and TNF-α levels indicates progressive, unmitigated inflammation. At the tissue level, this biochemical storm

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 May 2026 doi:10.20944/preprints202605.1119.v1

translates into a severe breakdown of the respiratory epithelium. The data specifically note “sloughing”, a process in which the protective outer layers of cells are shed, leaving the underlying tissue vulnerable and compromised.

In contrast, the phytochemical-based e-liquid appears to act as a biological buffer. Rather than following the linear escalation observed in the cigarette smoke group, these markers stabilize or decline over time. This suggests that the phytochemicals effectively "dampen" the pro-inflammatory signal, preventing it from reaching the damaging thresholds observed in traditional smoke exposure. The narrative of the data suggests that this dampening effect is not merely passive but is driven by active cellular defense mechanisms. The phytochemicals appear to upregulate antioxidant pathways, specifically the Nrf2 (Nuclear factor erythroid 2-related factor 2) pathway. By activating Nrf2, the cells increase their production of endogenous antioxidants, creating a more resilient internal environment.

This molecular strengthening correlates directly with the preservation of Epithelial Integrity. While the CS group experiences tissue breakdown, the phytochemical group maintains “Tight Junctions”. These junctions act as the “mortar” between cellular “bricks”, ensuring that the epithelial barrier remains leak-proof and structurally sound. Consequently, the phytochemical-based aerosol doesn't just reduce inflammation; it actively fortifies the respiratory system’s physical barriers against environmental stress.

Phytochemicals such as nicotine and ascorbic acid in aerosols can potentially interfere with signaling pathways. By neutralizing ROS before they trigger the NFκB switch, these aerosols may exhibit a blunted temporal inflammatory curve compared to the sharp spike seen with traditional smoking. The clinical significance of why ‘temporal’ matters, based on an understanding of timing, is crucial for intervention. If the IL-6/TNF-α axis is suppressed early on through phytochemical-based delivery, irreversible structural damage, such as the transition from bronchitis to full-blown emphysema, might be delayed or mitigated. However, it is vital to note that “less harmful” does not mean “safe”, as the long-term temporal effects of inhaling aerosolized phytochemicals are still being mapped in human cohorts.

4.7. Limitations

The study identifies several limitations related to its methodology, timeframe, and biological markers. Methodological and statistical limitations consists of; a) sample size, that may have lacked the statistical power required to detect significant differences in certain markers, specifically regarding lipid damage, b) study duration for the 12-week timeframe is identified as potentially insufficient to capture permanent, measurable lipid damage or to fully map the transition into long-term tobacco-related illnesses like COPD or emphysema, c) lack of specific cellular data, showed that the study's histopathological findings could not be supported by data regarding the distribution of inflammatory cells or specific damage to alveolar lining cells.

Biomarker and pathological constraints indicate that an incomplete inflammatory profile, based on IL-6 or TNF-α alone, does not provide a complete picture of organ-specific damage. IL-6 levels may be temporarily similar between substances (e.g., e-ascorbic acid vs. cigarettes), but this does not account for other types of damage, such as DNA damage or cancer, which one substance might cause and the other might not. A drop in TNF-α levels at 12 weeks could be misleading; it may indicate immune exhaustion (the body being unable to keep up production) rather than a healthy transition or resolution of inflammation. Besides, there was no significant variation in MDA levels across the groups. This is considered "unusual" in chronic smoke-exposure models, where MDA typically rises as antioxidant defenses decline.

The scope and generalizability revealed in this research pose risks to delivery methods. While some substances, such as ascorbic acid, are typically anti-inflammatory, the study highlights that their chemical properties may change or degrade upon heating and vaporization, potentially acting as pro-oxidants. However, the long-term human impact stated that the long-term temporal effects of inhaling aerosolized phytochemicals in humans are still not fully understood or mapped.

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 May 2026 doi:10.20944/preprints202605.1119.v1

5. Conclusions

The research concludes that both traditional cigarette smoke and e-liquid aerosols, including those containing phytochemicals such as ascorbic acid, induce significant systemic inflammation and antioxidant depletion over time. The study highlights a "temporal" shift in the body's response, moving from an acute defensive phase to a chronic, potentially damaging state. The inflammatory and antioxidant axis proved the transition to chronic inflammation. Exposure triggers a shift from an acute response (dominated by TNF-α at 8 weeks) to a chronic systemic inflammatory state (marked by a significant spike in IL-6 at 12 weeks). Antioxidant exhaustion occurred, while the body may initially increase antioxidant activity as a protective measure (oxidative eustress); prolonged 12-week exposure eventually overwhelms and depletes natural defenses like SOD3. The general irritant theory was supported by the study, which suggests that the physical state of the substance (aerosol/vapor) may be as problematic as its chemical composition; even "neutral" or "healthy" bases like propylene glycol and vegetable glycerin can trigger immune responses.

The pathological and metabolic impact caused by respiratory tissue degradation. Long-term exposure leads to structural damage, including reduced goblet cell density and thinning of the tracheal mucosal epithelium, thereby compromising respiratory defenses. The metabolic suppression, reflected in chronic inflammation, particularly the rise in IL-6, is strongly correlated with significantly reduced weight gain, suggesting that these inhaled insults inhibit normal growth or cause metabolic cachexia. The paradoxical effects of ascorbic acid were demonstrated: although typically an antioxidant, e-ascorbic acid delivered via vaporization elicited IL-6 levels similar to those of cigarette smoke. This suggests that the aerosolization process may negate its benefits or cause the substance to act as a pro-oxidant. Despite the risks, the research indicates that specific phytochemical-based aerosols might act as a biological buffer by upregulating antioxidant pathways (like Nrf2) and helping maintain epithelial integrity better than nicotine-only groups.

Finally, the study establishes that "less harmful" does not mean "safe". Body weight differentiation and tissue damage serve as visible markers of the internal struggle between oxidative stress and the body's inflammatory defense system. The novelty of this research lies in its temporal analysis of the body's transition from acute to chronic injury and its investigation of the paradoxical effects of aerosolized antioxidants.

Supplementary Materials: The following supporting information can be downloaded at: https://drive.google.com/drive/folders/1hDxqLrGeTYMl2iSPboK4himGbllNSoUi?usp=sharing.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used: "Conceptualization, A.P.; methodology, D.M.; software, L.A.A.; validation, N.G.S., A.W.P.Y., and M.M.S.; formal analysis, F.R.; investigation, H.I. and U.S.; resources, A.P.; data curation, D.M. and L.A.A.; writing—original draft preparation, A.P. and F.N.K.; writing—review and editing, D.M. and L.A.A.; visualization, H.I. and U.S.; supervision, U.S.; project administration, D.M.; funding acquisition, L.A.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Faculty of Medicine, Universitas Diponegoro, grant number: O1/UN7.F4lPPil142024, and "The APC was funded by the authors.".

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Health Research Ethics Commission of the Faculty of Medicine, Diponegoro University, in No. 088/EC-H/KEPK/FK-UNDIP/VIII/2024, Approval Date : August 07, 2024.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors would like to express their gratitude to the Faculty of Medicine, Universitas Diponegoro, for providing the administrative and technical support necessary to conduct this research. We also thank the laboratory staff and colleagues at the Biology Laboratory at Semarang State University, the Animal

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 May 2026 doi:10.20944/preprints202605.1119.v1

Laboratory of the Faculty of Medicine, Universitas Diponegoro, and Diponegoro National Hospital (RSND) [Department of Anatomy Pathology] for their assistance with data collection and materials.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

IL-6	Interleukin-6
TNF-α	Tumor Necrosis Factor-alpha
IL-1β	Interleukin-1-beta
IL-8	Interleukin-8
SOD-3	Superoxide dismutase-3
MDA	Malondialdehyde
AA	Ascorbic acid
ARRIVE	Animal Research: Reporting In Vivo Experiments
NMC	Nasal mucociliary clearance
GCD	Goblet Cells Density
ANOVA	Analysis of variance
H&E	Hematoxylin & Eosin
EDTA	Ethylenediaminetetraacetic acid
IACUC	Institutional Animal Care and Use Committee
COPD	Chronic obstructive pulmonary diseases
OD	Optical density

References

1. Dai X, Gakidou E, Lopez AD. Evolution of the global smoking epidemic over the past half century: Strengthening the evidence base for policy action. *Tob Control*. 2022;31(2):129-137. doi:10.1136/tobaccocontrol-2021-056535
2. Jerzyński T, Stimson G V., Shapiro H, Król G. Estimation of the global number of e-cigarette users in 2020. *Harm Reduct J*. 2021;18(1):1-10. doi:10.1186/s12954-021-00556-7.
3. Jerzyński T, Stimson G V. Estimation of the global number of vapers: 82 million worldwide in 2021. Drugs, Habits Soc Policy. 2023;24(2):91-103. doi:10.1108/DHS-07-2022-0028.
4. Wilson N, Summers JA, Ouakrim DA, Hoek J, Edwards R, Blakely T. Correction: Improving on estimates of the potential relative harm to health from using modern ENDS (vaping) compared to tobacco smoking. BMC Public Health. 2022;22(1):1-15. doi:10.1186/s12889-022-13983-3.
5. Goniewicz ML, Smith DM, Edwards KC, Blount BC, Caldwell KL, Feng J, Wang L, Christensen C, Ambrose B, Borek N, van Bemmel D, Konkel K, Erives G, Stanton CA, Lambert E, Kimmel HL, Hatsukami D, Hecht SS, Niaura RS, Travers M, Lawrence C, Hyland AJ. Comparison of Nicotine and Toxicant Exposure in Users of Electronic Cigarettes and Combustible Cigarettes. JAMA Netw Open. 2018 Dec 7;1(8):e185937. doi: 10.1001/jamanetworkopen.2018.5937.
6. Lerner CA, Sundar IK, Yao H, Gerloff J, Ossip DJ, McIntosh S, Robinson R, Rahman I. Vapors produced by electronic cigarettes and e-juices with flavorings induce toxicity, oxidative stress, and inflammatory response in lung epithelial cells and in mouse lung. PLoS One. 2015 Feb 6;10(2):e0116732. doi: 10.1371/journal.pone.0116732. PMID: 25658421; PMCID: PMC4319729.
7. Marques P, Piqueras L, Sanz MJ. An updated overview of e-cigarette impact on human health. *Respir Res*. 2021;22(1):1-14. doi:10.1186/s12931-021-01737-5.
8. Yu X, Zeng X, Xiao F, Chen R, Sinharoy P, Gross ER. E-cigarette aerosol exacerbates cardiovascular oxidative stress in mice with an inactive aldehyde dehydrogenase 2 enzyme. Redox Biol. 2022 Aug;54:102369. doi: 10.1016/j.redox.2022.102369. Epub 2022 Jun 14. PMID: 35751982; PMCID: PMC9243159.
9. Mishra A, Chaturvedi P, Datta S, Sinukumar S, Joshi P, Garg A. Harmful effects of nicotine. *Indian J Med Paediatr Oncol*. 2015;36(1):24-31. doi:10.4103/0971-5851.151771.

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 May 2026 doi:10.20944/preprints202605.1119.v1

10. Caliri A., Tomnasi S, Besarantina A. Relationships among smoking, oxidative stress, inflammation, macromolecular damage, and cancer Andrew. *Mutat Res Mutat Res*. 2021;787:108365. doi:doi:10.1016/j.mrrev.2021.108365.
11. Amjad MA, Ocazionez Trujillo D, Estrada-Y-Martin RM, Cherian SV. E-Cigarette or Vaping Product Use-Associated Lung Injury: A Comprehensive Review. *International Journal of Environmental Research and Public Health*. 2025; 22(5):792. https://doi.org/10.3390/ijerph22050792.
12. Jorge A Masso-Silva, Min Kwang Byun, Laura E Crotty Alexander, Acute and chronic effects of vaping electronic devices on lung physiology and inflammation, Current Opinion in Physiology. 2021; 22:100447. https://doi.org/10.1016/j.cophys.2021.06.001.
13. Yang X, Che W, Zhang L, Zhang H, Chen X. Chronic airway inflammatory diseases and e-cigarette use: a review of health risks and mechanisms. Eur J Med Res. 2025 Apr 1;30(1):223. doi: 10.1186/s40001-025-02492-9. PMID: 40170170; PMCID: PMC11959776.
14. Worden CP, Hicks KB, Hackman TG, Yarbrough WG, Kimple AJ, Farzal Z. The Toxicological Effects of e-Cigarette Use in the Upper Airway: A Scoping Review. Otolaryngol Head Neck Surg. 2024 May;170(5):1246-1269. doi: 10.1002/ohn.652. Epub 2024 Feb 14. PMID: 38353408; PMCID: PMC11060921.
15. Raduka A, Gao N, Chatburn RL, Rezaee F. Electronic cigarette exposure disrupts airway epithelial barrier function and exacerbates viral infection. Am J Physiol Lung Cell Mol Physiol. 2023 Nov 1;325(5):L580-L593. doi: 10.1152/ajplung.00135.2023. Epub 2023 Sep 12. PMID: 37698113; PMCID: PMC11068398.
16. Barrow, R.E., Wang, CZ., Cox, R.A. *et al*. Cellular sequence of tracheal repair in sheep after smoke inhalation injury. *Lung* **170**, 331–338 (1992). https://doi.org/10.1007/BF00177579.
17. Li C and Guan T (2025) Pulmonary function and physical performance in adolescent e-cigarette users: a narrative review. Front. Public Health 13:1703712. doi: 10.3389/fpubh.2025.1703712.
18. Allbright K, Villandre J, Crotty Alexander LE, Zhang M, Benam KH, Evankovich J, Königshoff M, Chandra D. The paradox of the safer cigarette: understanding the pulmonary effects of electronic cigarettes. Eur Respir J. 2024 Jun 28;63(6):2301494. doi: 10.1183/13993003.01494-2023. PMID: 38609098; PMCID: PMC12077657.
19. Rezk-Hanna M, Seals DR, Rossman MJ, Gupta R, Nettle CO, Means A, Dobrin D, Cheng CW, Brecht ML, Mosenifar Z, Araujo JA, Benowitz NL. Ascorbic Acid Prevents Vascular Endothelial Dysfunction Induced by Electronic Hookah (Waterpipe) Vaping. J Am Heart Assoc. 2021 Feb;10(5):e019271. doi: 10.1161/JAHA.120.019271. Epub 2021 Feb 20. PMID: 33615833; PMCID: PMC8174254.
20. Prasetyo A, Irawati N. Inhalation of Ascorbic Acid Modulates Sinonasal Immune System [Internet]. Ascorbic Acid - Biochemistry and Functions. IntechOpen; 2024. https://doi.org/10.5772/intechopen.110891.
21. Arumugam M, Sundaram RL, Jayajothi V, Arul M, Suresh JA, Kesavan SK. Recirculating bioavailable nicotine metabolite using ascorbic acid: A pragmatic approach for treating nicotine dependence. *Adv Redox Res*. 2024;10(October 2023). doi:10.1016/j.arres.2023.100089.
22. Ranjit Ambad, Saurabh Hadke, Pradip Jain, Ashish Anjankar. Role of Serum Malondialdehyde (MDA) and Vitmin- C Level in Non-Smokers and Chronic Smokers with Acute Myocardial Infarction (AMI) in Male. *Indian J Forensic Med Toxicol*. 2021;15(2):4363-4367. doi:10.37506/ijfmt.v15i2.15049.
23. K. E.Evoy, E. S.Yett, O.Ramey, and N.Lodise, "Evidence Regarding E-Cigarettes as a Harm Reduction Strategy and Treatment for E-Cigarette Cessation: An Overview and Guide for Clinicians," Journal of the American College of Clinical Pharmacy8, no. 9 (2025): 895–905, https://doi.org/10.1002/jac5.70092.
24. Nguyen NH, Tran GB, Nguyen CT. Anti-oxidative effects of superoxide dismutase 3 on inflammatory diseases. *J Mol Med*. 2020;98(1):59-69. doi:10.1007/s00109-019-01845-2.
25. Passi M, Shahid S, Chockalingam S, Sundar IK, Packirisamy G. Conventional and nanotechnology-based approaches to combat chronic obstructive pulmonary disease: Implications for chronic airway diseases. *Int J Nanomedicine*. 2020;15:3803-3826. doi:10.2147/IJN.S242516.
26. Morales M, Munné-Bosch S. Malondialdehyde: Facts and artifacts. *Plant Physiol*. 2019;180(3):1246-1250. doi:10.1104/pp.19.00405.
27. Toto A, Wild P, Graille M, et al. Urinary Malondialdehyde (MDA) Concentrations in the General Population—A Systematic Literature Review and Meta-Analysis. *Toxics*. 2022;10(4). doi:10.3390/toxics10040160.

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 May 2026 doi:10.20944/preprints202605.1119.v1

28. Saeed ZH, El Hakim MAEA, Mohamed NR. Chronic obstructive pulmonary disease in non-smokers: role of oxidative stress. *Egypt J Bronchol*. 2021;15(1). doi:10.1186/s43168-021-00088-5.
29. Son ES, Park JW, Kim YJ, et al. Effects of antioxidants on oxidative stress and inflammatory responses of human bronchial epithelial cells exposed to particulate matter and cigarette smoke extract. *Toxicol Vitr*. 2020;67(January). doi:10.1016/j.tiv.2020.104883.
30. Prasetyo A, Sadhana U, Budiman J. Nasal mucociliary clearance in smokers: A systematic review. *Int Arch Otorhinolaryngol*. 2021;25(1):160-169. doi:10.1055/s-0040-1702965.
31. Banerjee D, Kaul D. Combined inhalational and oral supplementation of ascorbic acid may prevent influenza pandemic emergency: A hypothesis. *Nutrition*. 2010;26(1):128-132. doi:10.1016/j.nut.2009.09.015.
32. Percie du Sert N, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, et al. (2020) Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. PLoS Biol 18(7): e3000411. https://doi.org/10.1371/ journal.pbio.3000411.
33. Charan J, Kantharia ND. How to calculate sample size in animal studies?. J Pharmacol Pharmacother 2013;4:303-6. Source of Support: Nil, Conflict of Interest: None declared.
34. Hage AN, Krause W, Mathues A, et al. Comparing the Effects of Electronic Cigarette Vapor and Cigarette Smoke in a Novel In Vivo Exposure System. *J Vis Exp*. 2017;(123):55672. Published 2017 May 24. doi:10.3791/55672.
35. Rahman, M. S., Sultana, K. R., Cho, Y., Yumshtyk, A., Kaya, T., & Matida, E. (2024). Effects of heating power and nicotine concentration on aerosol size distribution of a vaping device. *Aerosol Science and Technology*, 58(7), 706–718.
36. Harris E, Easter M, Ren J, Krick S, Barnes J, Rowe SM. An ex vivo rat trachea model reveals abnormal airway physiology and a gland secretion defect in cystic fibrosis. PLoS One. 2023 Oct 24;18(10):e0293367. doi: 10.1371/journal.pone.0293367. PMID: 37874846; PMCID: PMC10597513.
37. Yu, X. Y., Song, P., & Zou, M. H. (2018). Obesity Paradox and Smoking Gun. *Circulation Research*, *122*(12), 1642–1644. https://doi.org/10.1161/circresaha.118.312897.
38. Driva, S., Korkontzelou, A., Tonstad, S., Tentolouris, N., & Katsaounou, P. (2022). The Effect of Smoking Cessation on Body Weight and Other Metabolic Parameters with Focus on People with Type 2 Diabetes Mellitus. *International Journal of Environmental Research and Public Health*, *19*(20), 13222. https://doi.org/10.3390/ijerph192013222.
39. Chen, H., Hansen, M. J., Jones, J. E., Vlahos, R., Anderson, G. P., & Morris, M. J. (2008). Long-term cigarette smoke exposure increases uncoupling protein expression but reduces energy intake. *Brain Research*, *1228*, 81–88. https://doi.org/10.1016/j.brainres.2008.06.067.
40. Kiecolt-Glaser JK, Preacher KJ, MacCallum RC, Atkinson C, Malarkey WB, Glaser R. Chronic stress and age-related increases in the proinflammatory cytokine IL-6. *Proc Natl Acad Sci U S A*. 2003;100(15):9090-9095. doi:10.1073/pnas.1531903100.
41. Schichlein KD, Smith GJ, Jaspers I. Protective effects of inhaled antioxidants against air pollution-induced pathological responses. *Respir Res*. 2023;24(1):187. Published 2023 Jul 13. doi:10.1186/s12931-023-02490-7.
42. Gęgotek A, Skrzydlewska E. Antioxidative and Anti-Inflammatory Activity of Ascorbic Acid. *Antioxidants (Basel)*. 2022;11(10):1993. Published 2022 Oct 7. doi:10.3390/antiox11101993.
43. Tamagawa E, Bai N, Morimoto K, et al. Particulate matter exposure induces persistent lung inflammation and endothelial dysfunction. *Am J Physiol Lung Cell Mol Physiol*. 2008;295(1):L79-L85. doi:10.1152/ajplung.00048.2007.
44. Ohlson CG, Berg P, Bryngelsson IL, Elihn K, Ngo Y, Westberg H, Sjögren B. Inflammatory markers and exposure to occupational air pollutants. Inhal Toxicol. 2010 Nov;22(13):1083-90. doi: 10.3109/08958378.2010.520356. Epub 2010 Oct 29. PMID: 21029032.
45. Sussan TE, Gajghate S, Thimmulappa RK, Ma J, Kim J-H, Sudini K, et al. (2015) Exposure to Electronic Cigarettes Impairs Pulmonary Anti-Bacterial and Anti-Viral Defenses in a Mouse Model. PLoS ONE 10(2): e0116861. https://doi.org/10.1371/journal.pone.0116861.
46. Silva ALOD. E-cigarettes with vitamins and nutrients: where quackery and technology meet. Cigarros eletrônicos com vitaminas e nutrientes: onde o charlatanismo e tecnologia se encontram. *Cad Saude Publica*. 2024;40(1):e00024223. Published 2024 Feb 2. doi:10.1590/0102-311XPT024223.

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 May 2026 doi:10.20944/preprints202605.1119.v1

47. Aldaham S, Foote JA, Chow HH, Hakim IA. Smoking Status Effect on Inflammatory Markers in a Randomized Trial of Current and Former Heavy Smokers. *Int J Inflam*. 2015;2015:439396. doi:10.1155/2015/439396.
48. Débora Pez Jaeschke, Ligia Damasceno Ferreira Marczak, Giovana Domeneghini Mercali, Evaluation of non-thermal effects of electricity on ascorbic acid and carotenoid degradation in acerola pulp during ohmic heating, Food Chemistry, Volume 199, 2016, Pages 128-134, ISSN 0308-8146, https://doi.org/10.1016/j.foodchem.2015.11.117.
49. Shehata SA, Toraih EA, Ismail EA, Hagras AM, Elmorsy E, Fawzy MS. Vaping, Environmental Toxicants Exposure, and Lung Cancer Risk. *Cancers (Basel)*. 2023;15(18):4525. Published 2023 Sep 12. doi:10.3390/cancers15184525.
50. Delavallée, L., Semerano, L., Assier, E. *et al.* Active immunization to tumor necrosis factor-α is effective in treating chronic established inflammatory disease: a long-term study in a transgenic model of arthritis. *Arthritis Res Ther* **11**, R195 (2009). https://doi.org/10.1186/ar2897.
51. García-Domínguez M. The Role of TNF-α in Neuropathic Pain: An Immunotherapeutic Perspective. *Life (Basel)*. 2025;15(5):785. Published 2025 May 14. doi:10.3390/life15050785.
52. Dao* BN, Le HD T, Nguyen* KX, Viet TT, Tran CT, Luong TC et al. Relationship between serum TNF-α, IL-6, and IL- 10 levels and disease severity, and changes in the cytokines after treatment in patients with bacterial community-acquired pneumonia. Pneumon. 2023;36(4):27. https://doi.org/10.18332/pne/170181.
53. Gong X, Han Z, Fan H, et al. The interplay of inflammation and remodeling in the pathogenesis of chronic rhinosinusitis: current understanding and future directions. *Front Immunol*. 2023;14:1238673. Published 2023 Sep 12. doi:10.3389/fimmu.2023.1238673.
54. Landén NX, Li D, Ståhle M. Transition from inflammation to proliferation: a critical step during wound healing. *Cell Mol Life Sci*. 2016;73(20):3861-3885. doi:10.1007/s00018-016-2268-0.
55. Caliri AW, Tommasi S, Besaratinia A. Relationships among smoking, oxidative stress, inflammation, macromolecular damage, and cancer. *Mutat Res Rev Mutat Res*. 2021;787:108365. doi:10.1016/j.mrrev.2021.108365.
56. Osier W, Nozik ES, Sul C. Extracellular Superoxide Dismutase in Acute Respiratory Distress Syndrome: Pathogenic Mechanisms and Therapeutic Implications. *Antioxidants (Basel)*. 2026;15(2):249. Published 2026 Feb 13. doi:10.3390/antiox15020249.
57. Tang LP, Liu YL, Zhang JX, Ding KN, Lu MH, He YM. Heat stress in broilers of liver injury effects of heat stress on oxidative stress and autophagy in liver of broilers. *Poult Sci*. 2022;101(10):102085. doi:10.1016/j.psj.2022.102085.
58. Kumboyono K, Chomsy IN, Hakim AK, et al. Detection of Vascular Inflammation and Oxidative Stress by Cotinine in Smokers: Measured Through Interleukin-6 and Superoxide Dismutase. *Int J Gen Med*. 2022;15(September):7319-7328. doi:10.2147/IJGM.S367125.
59. Khudhur ZO, Smail SW, Awla HK, et al. The effects of heavy smoking on oxidative stress, inflammatory biomarkers, vascular dysfunction, and hematological indices. *Sci Rep*. 2025;15(1):1-15. doi:10.1038/s41598-025-03075-8.
60. Bezerra FS, Lanzetti M, Nesi RT, et al. Oxidative Stress and Inflammation in Acute and Chronic Lung Injuries. *Antioxidants*. 2023;12(3):1-46. doi:10.3390/antiox12030548.
61. Jebur HB, Saray BAAK Al, Idbeis HI, et al. Lung Histo-Pathological Changes and Sods Gene Expression in Wister Rats Exposed To Conventional and Electronic Cigarettes. *Bull Pharm Sci Assiut*. 2023;46(2):1429-1440. doi:10.21608/BFSA.2023.327634.
62. Lim EY, Kim G-D. Particulate Matter-Induced Emerging Health Effects Associated with Oxidative Stress and Inflammation. *Antioxidants*. 2024; 13(10):1256. https://doi.org/10.3390/antiox13101256.
63. Gęgotek A, Skrzydlewska E. Antioxidative and Anti-Inflammatory Activity of Ascorbic Acid. *Antioxidants*. 2022;11(10). doi:10.3390/antiox11101993.
64. Ali A, Riaz S, Khalid W, et al. Potential of ascorbic acid in human health against different diseases: an updated narrative review. *Int J Food Prop*. 2024;27(1):493-515. doi:10.1080/10942912.2024.2327335.
65. Li X, Yang T, Sun Z. Hormesis in Health and Chronic Diseases. *Trends Endocrinol Metab*. 2019;30(12):944-958. doi:10.1016/j.tem.2019.08.007.

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 May 2026 doi:10.20944/preprints202605.1119.v1

66. Mustafa Cemre Sonmez, Rengin Ozgur, Baris Uzilday, Reactive oxygen species: Connecting eustress, hormesis, and allostasis in plants, Plant Stress, Volume 8, 2023, 100164, ISSN 2667-064X, https://doi.org/10.1016/j.stress.2023.100164. (https://www.sciencedirect.com/science/article/pii/S2667064X23000325).
67. Rahman M, Sompa SI, Introna M, Upadhyay S, Ganguly K, Palmberg L. Lipid from electronic cigarette-aerosol both with and without nicotine induced pro-inflammatory macrophage polarization and disrupted phagocytosis. *J Inflamm (Lond)*. 2023;20(1):39. Published 2023 Nov 17. doi:10.1186/s12950-023-00367-6.
68. Roslan M, Milewska K, Szoka P, Warpechowski K, Milkowska U, Holownia A. Diverse Impact of E-Cigarette Aerosols on Oxidative Stress and Inflammation in Lung Alveolar Epithelial Cells (A549). *International Journal of Molecular Sciences*. 2025; 26(22):10967. https://doi.org/10.3390/ijms262210967.
69. Mokrá D. Acute lung injury - from pathophysiology to treatment. *Physiol Res*. 2020;69(Suppl 3):S353-S366. doi:10.33549/physiolres.934602.
70. Rigden HM, Alias A, Havelock T, et al. Squamous Metaplasia Is Increased in the Bronchial Epithelium of Smokers with Chronic Obstructive Pulmonary Disease. PLoS One. 2016;11(5):e0156009. Published 2016 May 26. doi:10.1371/journal.pone.0156009..
71. Rathnayake SNH, Ditz B, van Nijnatten J, et al. Smoking induces shifts in cellular composition and transcriptome within the bronchial mucus barrier. *Respirology*. 2023;28(2):132-142. doi:10.1111/resp.14401.
72. Pinkston RI, Schexnayder M, Perveen Z, et al. MMP12 deficiency attenuates menthol e-cigarette plus house dust-mite effects on pulmonary iron homeostasis and oxidative stress. *Respir Res*. 2025;26(1). doi:10.1186/s12931-025-03213-w.
73. Kato K, Chang EH, Chen Y, et al. MUC1 contributes to goblet cell metaplasia and MUC5AC expression in response to cigarette smoke in vivo. *Am J Physiol - Lung Cell Mol Physiol*. 2020;319(1):L82-L90. doi:10.1152/ajplung.00049.2019.
74. Ravi R, Pal I, Kumar S, Babu AS, Halder I, Roy S. Correlation between Upper Airway and Lower Airway Function in Current Smokers, Never Smokers and Former Smokers. *J Adv Med Med Res*. 2023;35(19):125-148. doi:10.9734/jammr/2023/v35i195148.
75. Kwan D, Ahmed S, Ahmed AR. The effects of electronic cigarettes on the respiratory tract: A review of case reports. *Adv Biomed Heal Sci*. 2025;4(4):162-172. doi:10.4103/abhs.abhs_103_24.
76. Jiang C, Chen Q, Xie M. Smoking increases the risk of infectious diseases: A narrative review. *Tob Induc Dis*. 2020;18:1-17. doi:10.18332/tid/123845.
77. Jagirdar RM, Grammatikopoulos A, Ioannou M, et al. Short Term Exposure of Sheep Tracheal Epithelium to Cigarette Smoke Extract Reduces ENaC Current: A Pilot Study. *In Vivo (Brooklyn)*. 2024;38(5):2294-2299. doi:10.21873/invivo.13694.
78. Sharma G, Pund S, Govindan R, et al. A Proteomics Investigation of Cigarette Smoke Exposed Wistar Rats Revealed Improved Anti-Inflammatory Effects of the Cysteamine Nanoemulsions Delivered via Inhalation. *Omi A J Integr Biol*. 2023;27(8):338-360. doi:10.1089/omi.2023.0074.
79. Wang J, Ding R, Liang J, Wang H, Wang P. Morphological Effects of Cigarette Smoking on Respiratory System and Related Functions Based on AQPs Expression. *Int J Morphol*. 2023;41(2):539-547. doi:10.4067/S0717-95022023000200539.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.