EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com
ISSN 2349-8870
Volume: 12
Issue: 3
Year: 2025

PROTECTIVE EFFECTS OF ALLIUM SATIVUM (GARLIC) AGAINST FORMALIN-INDUCED LUNG DAMAGE IN WISTAR RATS

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Article Received on 14/01/2025 **Article Revised on 04/02/2025** **Article Accepted on 24/02/2025**

ABSTRACT
Formalin is widely used for disinfection and embalming but is associated with respiratory complications due to prolonged inhalation. *Allium sativum* (garlic) contains bioactive compounds with antioxidant, anti-inflammatory, and antimicrobial properties. This study examined the protective effects of *Allium sativum* on formalin-induced lung damage in adult male Wistar rats. Twenty rats (100–120 g) were divided into five groups (A–E, four per group). Group A served as the control. Group B was exposed to 4 mL of 100% formalin. Group C received 500 mg/kg of *Allium sativum*. Groups D and E received 250 mg/kg and 500 mg/kg of *Allium sativum* with formalin exposure, respectively. Formalin exposure lasted 30 minutes daily, while garlic extract was administered orally for 28 days. Histological analysis revealed thrombus-like aggregates in blood vessels in Group B. Group D showed alveolar proliferation, thickened alveolar epithelium, and alveolar sac expansion, while Group E exhibited terminal bronchiole constriction. These findings suggest that formalin alters lung tissue structure, and *Allium sativum* exerts a protective effect, mitigating formalin-induced lung damage.

KEYWORDS: Formalin toxicity, *Allium sativum*, Lung protection, Oxidative stress.

INTRODUCTION
Garlic (*Allium sativum* L.) of the family Liliaceae is a perennial herb known for its distinctive pungent odor. It has been widely used as a food ingredient and spice since ancient times. Beyond its culinary applications, garlic is recognized for its numerous physiological benefits, including its potential role in the treatment of cardiovascular diseases[1] and hyperlipidemia.[2] The major bioactive components in garlic are sulfur compounds such as alliin, allicin, diallyl sulfide, diallyl disulfide, diallyl trisulfide, and S-allylcysteine (SAC).[3] Research has demonstrated that garlic extracts enhance T-cell cytotoxicity and lymphoproliferative capacity.[4] Additionally, garlic has shown protective effects against dust mite (*Dermatophagoides pteronyssinus*)-induced allergic airway inflammation in mice,[5] although the precise mechanisms and active compounds responsible remain unclear.

The garlic bulb, particularly its cloves, is the most commonly used part of the plant and is believed to possess multiple medicinal properties. These properties include regulating high blood pressure, lowering cholesterol levels, and serving as a remedy for the common cold.[6] Members of the *Allium* genus, including garlic, are rich in organosulfur compounds, which contribute to their health benefits.[7] However, some of these compounds have also been linked to toxicity in mammals.[8] Notably, N-propyl disulfide has been found to interfere with glucose-6-phosphate dehydrogenase activity in red blood cells, leading to oxidative damage to hemoglobin.[9] Ingesting onions has even been reported to cause hemolytic anemia.[10] Despite these concerns, garlic continues to be widely studied for its numerous health-promoting effects and is often regarded as a functional food with disease-preventative properties. This growing interest has led to the development of garlic-infused medicinal products.

Formaldehyde is a widely used chemical, with global production reaching millions of tons annually.[11] It is present in various industrial and consumer products and can also be generated as a byproduct of fires, cigarette smoke, and automotive exhaust.[12] The International Agency for Research on Cancer (IARC) classifies formaldehyde as a Group I carcinogen, linking it to various cancers.[13,14,11] Formaldehyde exposure has been associated with systemic and localized allergic reactions, including anaphylaxis, contact dermatitis, and respiratory tract irritation.[15,16] Inhalation of formaldehyde can lead to lung edema, bronchioalveolar constriction, and severe respiratory complications.[17] High concentrations of formaldehyde exposure have been shown to cause significant hyperplasia and squamous metaplasia of the nasal respiratory epithelium in animal studies.[12]

In modern embalming, formaldehyde is commonly used in the form of formalin, serving as both a disinfectant and preservative to maintain the structural integrity of deceased human bodies. Formalin is a liquid solution containing approximately 37% formaldehyde dissolved in water, with methanol added to prevent polymerization. While methanol and water reduce its volatility compared to pure formaldehyde, formalin readily releases formaldehyde fumes upon exposure to air.[18]

Formaldehyde in formalin reacts with proteins, particularly the amino (-NH_2) groups of amino acids in tissues, forming methylene bridges (-CH_2-) between protein molecules. This cross-linking stabilizes cellular structures, preventing enzymatic breakdown (Autolysis) and microbial decomposition. Methanol plays a crucial role in preventing polymerization, ensuring that formaldehyde remains in a usable liquid state.[18]

Given the known toxicity of formaldehyde and its potential effects on respiratory health, there is a need to explore protective interventions against formalin-induced lung damage. This study aims to evaluate the protective effects of *Allium sativum* (Garlic) on formalin-induced lung damage in adult male Wistar rats.

MATERIALS AND METHODS

Ethical Clearance, Location and Duration of Study

Required ethical approval for this study was obtained from the Ethical Committee, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State. The experiment was conducted at the Department of Anatomy, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State. The rats were acclimatized for two weeks before the test sample administration, which lasted for 28 days.

Animal Procurement, Care and Treatment

Twenty adult male Wistar rats (100g-120g) were purchased from the Department of Human Physiology, Nnamdi Azikiwe University, Nnewi Campus. The animals were housed in a well-ventilated facility under standard conditions (temperature: 27℃-31℃) and fed a standard rat diet with *ad libitum* access to distilled water. Cage bedding was changed every two days to maintain hygiene and prevent infections. All procedures adhered to the ethical guidelines of the Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, in compliance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.[19]

Procurement of Formalin, *Allium sativum* (Garlic) and Preparation of Extract

Formalin (Formaldehyde 40%, produced by Jubaili Agrotec) was purchased from Nkwo Market, Nnewi and reconstituted. Fresh cloves of garlic were procured from Eke Amaobi Market, Otolo Nnewi, Anambra State. These cloves were shade-dried and ground into coarse powder. Fifty grams (50g) of the powder was soaked in 250mL of absolute ethanol and allowed to macerate for 48 hours with intermittent shaking. The mixture was then filtered using porcelain cloth and subsequently with Whatman No. 1 filter paper. The filtrate (*allium sativum* extract) was evaporated to dryness using a rotary evaporator and stored in a refrigerator for later use.

Acute Toxicity Test (LD_{50}) of Allium Sativum Extract and Formalin

The acute toxicity tests of Allium Sativum Extract and Formalin was conducted following the Lorke method [20] in the Department of Human Physiology, Nnamdi Azikiwe University.

The LD_{50} of Allium Sativum was found to be above 5000 mg/kg BW via the oral route while that of formaldehyde inhalation was calculated as 19.36 mL in a 407×350 mm Inhalation Exposure Chamber.

Experimental Design and Protocol

Prior to the commencement of administration of substrates, the rats were weighed and randomly assigned to five groups (A–E), each consisting of four rats. Group A (Control) received distilled water and standard rat feed, Group B was exposed to formalin only, Group C received 500 mg/kg of Allium sativum daily, Group D received 250 mg/kg of Allium sativum along with formalin exposure, and Group E received 500 mg/kg of Allium sativum along with formalin exposure. Allium sativum was administered orally once daily immediately after formalin exposure. Using an Inhalation Exposure Chamber, the respective animals were subjected to 4 mL of formalin vapor for 30 minutes each day over a period of 28 days.

Termination of Experiment and Histopathological Evaluation

Sample Collection

Twenty-four hours after the last administration, the rats were anesthetized with chloroform vapor. Lung tissues were harvested, weighed, and fixed in 10% formal saline for histological studies.

Tissue processing

Tissue samples were processed using standard histological techniques. Fixation was performed in 10%

formal saline for three days, followed by dehydration using alcohol gradients from 50% to absolute alcohol for 30 minutes each. Clearing was achieved using xylene to remove alcohol, after which tissues were embedded in paraffin wax at 56°C and subsequently sectioned. Microtomy was conducted using a rotary microtome, cutting sections at 5 microns for staining. The Hematoxylin and Eosin (H&E) staining procedure involved dewaxing in xylene for 30 minutes, hydration through decreasing alcohol concentrations, hematoxylin staining for 20 minutes, differentiation in 1% acid alcohol, eosin counterstaining for five minutes, dehydration, and final clearing in xylene before mounting with D.P.X for microscopic examination. Digital photomicrographs were taken at ×400 magnification using OMAX software, with NIH Image software employed for quantitative analysis.

Data analysis
Data were analyzed using SPSS v27.0.1. Results were expressed as mean ± SEM. Organ weights were analyzed via one-way ANOVA followed by post hoc LSD multiple comparisons. Body weight data were analyzed using Student's t-test, with significance set at $P<0.05$.

RESULTS

Physical and Behavioral Changes
Following exposure to formalin, slow movement, staggering, and weakness were observed more frequently in Group C.

Effect of Ethanolic Extract of Allium Sativum and Formalin Fume on Body Weight of Experimental Animals
Findings as presented in Table 1.0 showed a significant increase in the mean body weight in Groups A, B, C, and D when comparing initial weight to final weight. However, Group E exhibited no significant differences but indicated an increase.

Table 1.0: Effect of Co-administration of Ethanolic extract of *Allium sativum* and formalin fumes on the bodyweights of Wistar rats.

Groups	Initial weight(g)	Final weight(g)	p-value	t-vale
	MEAN±SEM	MEAN±SEM		
Group A(control)	150.71±7.55	196.35±10.57	0.029*	-3.035
Group B(4mls/0.1m^3 of Formalin)	140.98±4.85	157.57±3.21	0.023*	-3.226
Group C(500mg/kg of EAS)	143.33±3.75	171.77±3.20	0.002*	-5.604
Group D(4mls/0.1m^{3}Fom.+250mg/kg of EAS)	148.52±11.32	176.34±3.15	0.035*	-3.122
Group E(4mls/0.1m^{3}Fom.+500mg/kg of EAS)	145.96±5.46	178.38±13.14	0.076#	-2.383

Data were subjected to a paired t-test, with significance indicated by (*) and non-significance (#) when initial weight was compared to final weight.

Relative lung weight observation
Findings as presented in Table 2.0 showed that there was a significant increase in the mean relative lung weight in Group B (p=0.012), while Groups C, D, and E (p=0.680, p=0.760, p=0.607) showed no significant difference compared to Group A, despite an increase in Groups C and D and a decrease in Group E. Additionally, Group B (p=0.026) exhibited a significant increase, whereas Groups D and E (p=0.913, p=0.361) showed no significant difference compared to Group C, with a decrease observed in Groups D and E.

Table 2.0: Effect of Co-administration of Ethanolic extract of *Allium sativum* and formalin fumes on the relative lungs weight of Wistar rats.

Groups	Relative lungs weight(g)
	MEAN±SEM
Group A(control)	1.83±0.12
Group B(4mls/0.1m^3 of Formalin)	2.44±0.07*a
Group C(500mg/kg of EAS)	1.92±0.17#
Group D(4mls/0.1m^3 Form.+250mg/kg of EAS)	1.89±0.07#b
Group E(4mls/0.1m^3 Form+500mg/kg of EAS)	1.73±0.19#b
P-value	0.038
F-ratio	3.855

Data were analyzed using ANOVA followed by post-hoc multiple LSD comparisons. Significance is denoted as (*), non-significance (#) compared to Group A; significance (a), non-significance (b) when compared to Group C.

Histopathological findings

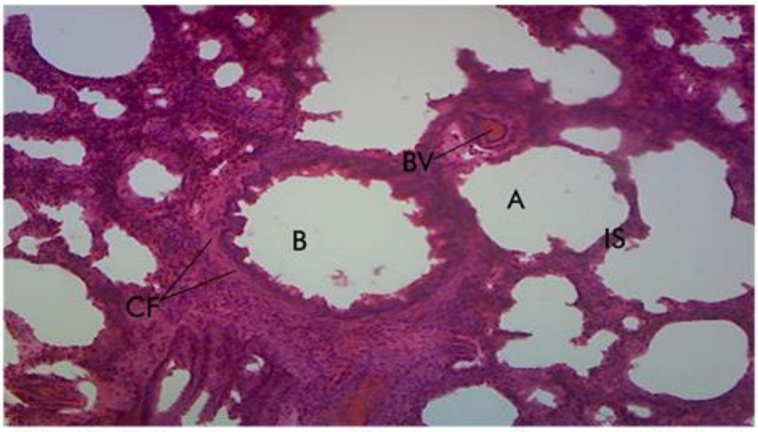

Plate 1: Photomicrograph of the Lungs of group A (Control) showing a normal histoarchitecture. With normal bronchiole (B), Alveoli (A), Alveolar sac (AS), Intra-alveolar septae (IS), Blood vessel (BV), Collagen fibers (CF) H&E×100.

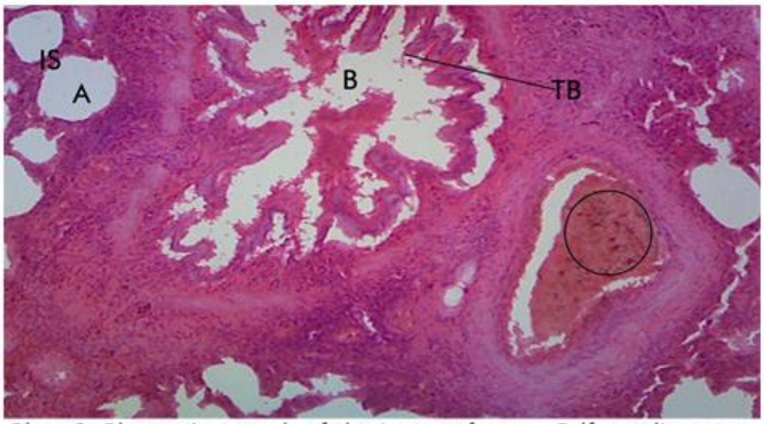

Plate 2: Photomicrograph of the Lungs of group B (formalin vapor only), Showing a histoarchitecture with thrombus-like aggregates in Blood vessels. B: Bronchiole, A: Alveoli, IS: Intra alveolar septae, BV: Blood vessel, CF: Collagen fibers, TB: Terminal bronchioles. H&E,×100.

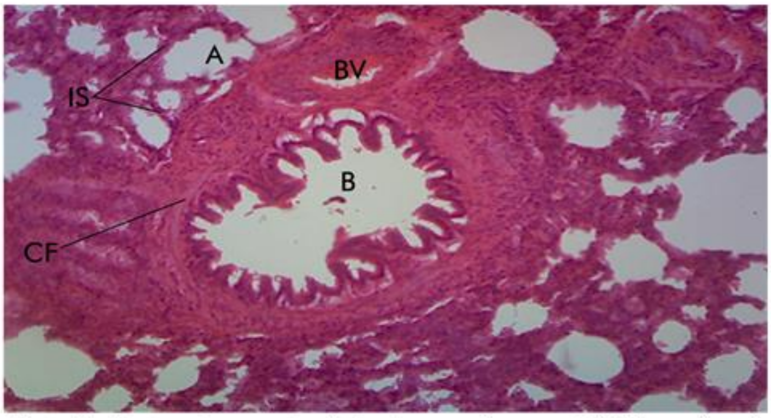

Plate 3: Photomicrograph of the Lungs of group C (500 mg/kg of Allium sativum), showing normal lung histoarchitecture :
B: Bronchiole, A: Alveoli, AS: Alveolar sac, IS: Intra-alveolar septae, BV: Blood vessel, CF: Collagen fibers. H&E,×100.

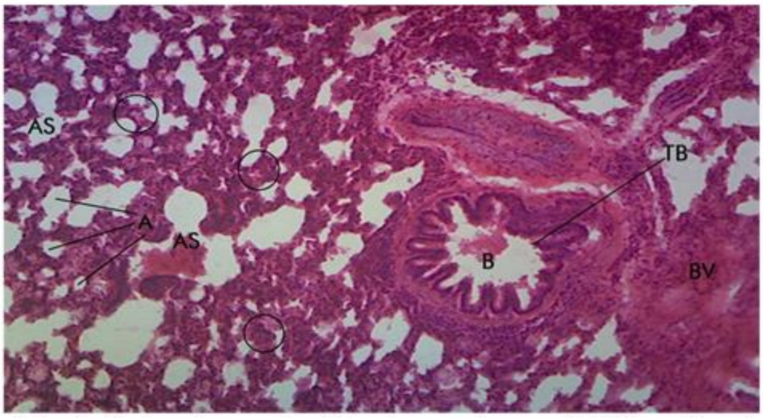

Plate 4: Photomicrograph of the Lungs of group D (250 mg/kg of *Allium sativum* along with formalin), showing histoarchitecture with proliferation of Alveoli and Alveolar sacs and noticeable thickening of Alveolar epithelium (circled). B: Bronchiole, A: Alveoli, AS: Alveolar sac, BV: Blood vessel; TB: Terminal bronchioles. H&E,×100.

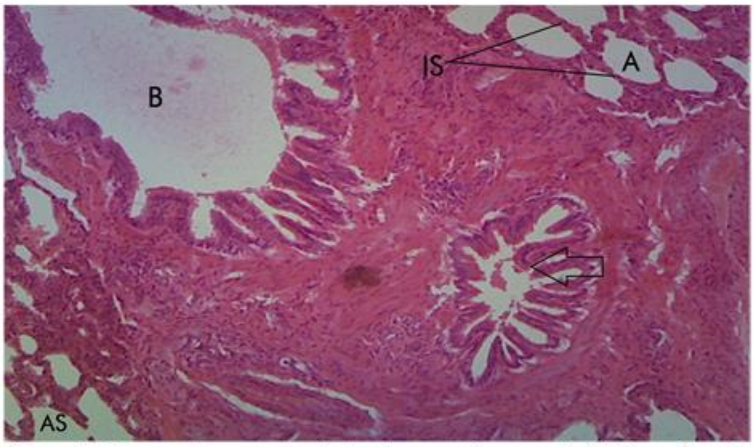

Plate 5: Photomicrograph of the Lungs of group E (500 mg/kg of *Allium sativum* along with formalin), showing constriction of Terminal bronchioles (arrow) B: Bronchiole, A: Alveoli, IS: Intra-alveolar septae, BV: Blood vessel. H&E,×100.

DISCUSSION

Formaldehyde (FA), a high-volume chemical, is widely found in the environment due to natural sources and human activities. Major indoor air sources include building materials such as wooden products, furniture, particleboard, plywood, and medium-density fiberboard, as well as consumer products and combustion processes.[21] Formalin has classified as a human carcinogen.[13,14]

Exposure to fumes of formalin has been documented to cause inflammatory changes in the lungs of the animals including congestion, alveoli filled with a homogeneous red-staining exudates and slight desquamation of the alveolar lining cells.[21,22] Formalin causes its damaging effects via complex processes such as activation of inflammatory pathways, oxidative stress, and immunological responses which includes generating reactive oxygen species (ROS) in respiratory tissues through multiple mechanisms, including mitochondrial dysfunction, NADPH oxidase activation, and depletion of antioxidant defenses.[23,24,25] Excessive ROS production depletes the body's natural antioxidant reserves, leading to oxidative damage in DNA, lipids, and proteins.[26] Furthermore, ROS-mediated oxidative stress disrupts cellular homeostasis and exacerbates inflammation by activating redox-sensitive transcription factors such as NF-κB and AP-1, which promote the expression of pro-inflammatory genes.[27]

Garlic (Allium sativum) has high medicinal value and is used to treat various human diseases. It has antibacterial,[28,29] antifungal,[30] and antiviral properties[31] and is widely used as a therapeutic agent. Researchers have explored garlic’s medicinal properties in treating cardiovascular diseases, cancer, diabetes, hypertension, atherosclerosis, and hyperlipidemia,[32] This study aimed to investigate the pulmonoprotective effects of Allium sativum against formalin-induced lung damage in adult male Wistar rats.

Results showed that exposure to both formalin and allium sativum didn't affect the body weight of the animals. However, experimental rats exposed to formalin alone had significantly higher relative lungs weight probably due to the accumulation of coagulated blood in the blood vessels as seen in plate 2. On the other hand, co-administration of formalin with allium sativum extract didn't cause any significant difference in lung weight when compared to that of Control group.

Histological examination showed that formalin fumes exposure caused significant lungs damage with thrombus-like aggregates in blood vessels. However, coadministration with allium sativum provided some level of protection with low dose of Allium sativum appearing to offer better protective effects suggesting that an optimal dosage range may be necessary to maximize its benefits while minimizing adverse effects.

Findings from previous researches has documented the presence of numerous healthy phytochemicals[33,34,35] which have enhanced the detoxification activity of the liver.[36] Some of its secondary metabolites exhibit strong antioxidant properties.[33,37,38] which may contribute to its protective effects on the lungs. However, previous in vivo studies have reported that prolonged consumption of high doses of raw garlic can lead to weight loss and anemia due to red blood cell (RBC) lysis, as well as stomach injuries.[8] Additionally, garlic has been shown to exert anti-androgenic effects by inhibiting spermatogenesis in rats, potentially leading to reproductive complications.[39]

CONCLUSION

This study demonstrates that exposure to formalin induces significant structural changes in lung tissue, including alveolar proliferation, thickened epithelium, and terminal bronchiole constriction. However, Allium sativum exhibits a protective effect against these formalin-induced alterations, potentially due to its antioxidant, anti-inflammatory, and antimicrobial properties. The varying degrees of lung tissue response across different treatment groups suggest a dose-dependent protective mechanism of garlic extract. While the findings highlight the therapeutic potential of Allium sativum in mitigating respiratory complications caused by formalin inhalation, further research is necessary to explore its precise mechanisms of action and potential clinical applications.

ACKNOWLEDGEMENT

We acknowledge the assistance of all the laboratory technologists in both Histology (Department of Anatomy) and Department of Human Physiology, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University for their help especially in animal handling and other laboratory exercises.

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