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Alpha-Lipoic Acid Preserves Bcl-2 Expression in Rabbit Lung Tissue During 30-Day Inhalation Exposure to the Pesticide Nurinol

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Abstract Pesticide-induced lung injury is closely associated with oxidative stress and apoptosis, particularly through the downregulation of mitochondrial anti-apoptotic proteins such as Bcl-2. This study aimed to evaluate the expression of Bcl-2 in rabbit lung tissue following 30-day inhalation exposure to Nurinol (chlorpyrifos- and cypermethrin-based pesticide) and to assess the protective role of alpha-lipoic acid. Male rabbits (Oryctolagus cuniculus) were divided into two groups (n = 16 each): Group 1 received Nurinol via aerosol (¾ LD_{50}, 216.8 mg/kg), and Group 2 received the same exposure with concurrent oral alpha-lipoic acid therapy (4.2 mg/kg/day). Lung samples were collected for histopathology and immunohistochemical analysis of Bcl-2. Group 1 showed severe epithelial damage, inflammation, and significantly reduced Bcl-2 expression (15.2% positive cells). In contrast, Group 2 displayed preserved lung architecture and a higher percentage of Bcl-2–positive cells (32.0%), with strong immunopositivity observed in over half the animals. These findings demonstrate that Nurinol suppresses Bcl-2 expression and promotes lung injury, while alpha-lipoic acid enhances Bcl-2-mediated anti-apoptotic protection. alpha-lipoic acid may offer a promising antioxidant strategy for mitigating acute pesticide-induced pulmonary toxicity.

Keywords Pesticide-induced lung injury, Bcl-2 expression, Alpha-lipoic acid, Oxidative stress, Apoptosis, Chlorpyrifos and cypermethrin, Immunohistochemistry

1. Introduction

Pesticide-induced pulmonary toxicity is a significant public health concern, particularly in agricultural regions where chronic exposure to organophosphate-based compounds is common. Numerous studies have demonstrated that inhalation or systemic absorption of pesticides leads to oxidative stress, mitochondrial dysfunction, and apoptosis in various organ systems, including the lungs [1]. The respiratory system, with its vast surface area and direct contact with environmental agents, is especially susceptible to reactive oxygen species (ROS)–mediated cellular damage [2].

A central mediator of cellular survival under toxic conditions is the Bcl-2 protein family, which includes both pro-apoptotic and anti-apoptotic members. Among these, Bcl-2 (B-cell lymphoma 2) functions as a key anti-apoptotic regulator that preserves mitochondrial membrane integrity and prevents the release of cytochrome c, apoptosis-inducing factor (AIF), and other pro-death molecules into the cytoplasm [3].

Experimental studies have confirmed that exposure to various pesticides, including chlorpyrifos, paraquat, and cypermethrin, downregulates Bcl-2 expression and promotes pro-apoptotic signaling in pulmonary tissues [4]. These molecular alterations correlated with structural lung damage, inflammation, and decreased respiratory function. Similar findings were reported in models of paraquat-induced lung fibrosis, where Bcl-2 suppression was tightly associated with fibroblast activation and alveolar cell apoptosis [5].

Alpha-lipoic acid is a well-characterized antioxidant that has shown promise in modulating mitochondrial homeostasis and apoptotic pathways under toxic conditions. As both a ROS scavenger and a cofactor in mitochondrial dehydrogenase complexes, alpha-lipoic acid plays a dual role in cellular defense—neutralizing oxidative intermediates and stabilizing mitochondrial bioenergetics [6]. Several animal studies have confirmed ALA's ability to restore Bcl-2 expression and reduce Bax translocation in organs subjected to oxidative injury [7].

Despite increasing recognition of the Bcl-2 pathway as a molecular switch between survival and death under toxic

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stress, limited studies have investigated its role specifically in pesticide-induced lung damage and the potential protective effects of antioxidants like alpha-lipoic acid in this context. Nurinol, a chlorpyrifos-based pesticide formulation with known oxidative properties, has been shown in preliminary studies to induce morphological disruption in bronchiolar epithelium and alveolar collapse in experimental animals [8]. However, the molecular determinants of this toxicity — particularly the status of Bcl-2 in lung cells—remain underexplored.

Therefore, the current study was designed to evaluate the immunohistochemical expression of Bcl-2 in lung tissue of rabbits exposed to Nurinol, and to determine how co-administration of alpha-lipoic acid modifies this expression.

2. Materials and Methods

Healthy adult male rabbits (Oryctolagus cuniculus), weighing 2.4–2.8 kg, were housed under standard laboratory conditions. A total of 64 animals were randomly divided into four experimental groups (n = 16 each):

Group 1: 30-day inhalation exposure to Nurinol

Group 2: 30-day inhalation exposure to Nurinol + alpha-lipoic acid (ALA) therapy

Exposure was conducted in a sealed 45-liter inhalation chamber (50×30×30 cm) equipped with a nebulizer delivering a fine aerosol at 0.5–0.7 mL/min. Nurinol, containing 55% active ingredients (chlorpyrifos and cypermethrin), was aerosolized to deliver a dose equivalent to ¾ LD_{50} (216.8 mg/kg) 10-minute exposures per day during 30. After each 10-minute exposure, the chamber was ventilated and animals returned to their cages.

In treated groups, alpha-lipoic acid (ALA) was administered daily via oral gavage at 4.2 mg/kg dissolved in saline, concurrently with pesticide exposure.

At the end of each exposure period (day 30), animals were euthanized under deep anesthesia. Lung tissues were harvested, fixed in 10% buffered formalin, and paraffin-embedded. Sections (4–5 μm) were stained with hematoxylin and eosin (H&E) for morphological evaluation.

Immunohistochemistry for Bcl-2

Bcl-2 expression was assessed using standard IHC. After antigen retrieval (citrate buffer, pH 6.0) and blocking, lung sections were incubated with a primary anti–Bcl-2 antibody, followed by a biotinylated secondary antibody and DAB chromogen. Hematoxylin counterstaining was performed. Positive and negative controls were included. Bcl-2 expression was evaluated semi-quantitatively (score 0–3) based on staining intensity and proportion of positive cells:

0 = Negative
1 = Weak
2 = Moderate
3 = Strong

3. Results

Histological Findings in 30-Day Exposure Groups. Histopathological examination of lung tissue from animals in Group 1 (30-day Nurinol exposure without treatment) revealed pronounced toxic-alterative changes in the respiratory portion of the lungs. The terminal bronchioles and surrounding alveolar epithelium demonstrated typical features of cellular damage, including cytoplasmic vacuolization, swelling, nuclear pyknosis, and desquamation of the epithelial lining. Infiltration of mononuclear inflammatory cells was noted in the peribronchiolar and interstitial spaces. In some samples, early fibrotic remodeling of the interalveolar septa was observed, indicating the onset of chronic tissue injury and compromised pulmonary architecture. These changes collectively suggest that inhalation exposure to Nurinol caused significant structural and functional injury, impairing respiratory epithelial integrity and promoting early inflammatory and degenerative responses.

In contrast, animals from Group 2, which received alpha-lipoic acid (ALA) antioxidant therapy during the same 30-day exposure period, exhibited markedly reduced tissue damage. The bronchiolar epithelium in these animals was generally preserved, with well-defined cell borders, intact cilia, and nuclei containing dispersed euchromatin, indicating active metabolic function. The alveolar septa appeared thin and structurally intact, with minimal inflammatory cell infiltration. Importantly, no features of necrosis, hemorrhage, or early fibrosis were observed in this group. These findings suggest that ALA conferred substantial histological protection against Nurinol-induced injury, most likely by stabilizing cellular membranes and reducing oxidative stress at the mitochondrial level.

Immunohistochemical Analysis of Bcl-2 Expression. Group 1 – 30-Day Nurinol Exposure without Antioxidant. Immunohistochemical staining for the anti-apoptotic protein Bcl-2 in Group 1 animals showed a predominantly low level of expression across the lung tissue. Digital image analysis using QuPath software revealed that out of 593 identified cells, only 90 cells stained positively for Bcl-2, representing approximately 15.2% of the total population. Positive staining was generally weak, appearing as faint cytoplasmic or membranous brown coloration in scattered bronchiolar epithelial cells.

Semi-quantitative scoring showed that:

11 animals (68.75%) demonstrated score 1
(weak expression),
2 animals (12.5%) had score 2 (moderate expression),
and 5 animals (31.25%) showed score 0 (no detectable expression).

No animals in this group showed strong Bcl-2 expression (score 3).

These results indicate that 30-day inhalation of Nurinol alone leads to significant suppression of Bcl-2, reflecting an impaired anti-apoptotic defense. The predominance of weak

or absent expression suggests that the cells were unable to effectively resist apoptotic or necrotic stimuli, which aligns with the observed histopathological damage. This insufficient Bcl-2 response likely contributes to enhanced epithelial vulnerability, cell loss, and progression of inflammatory and degenerative processes.

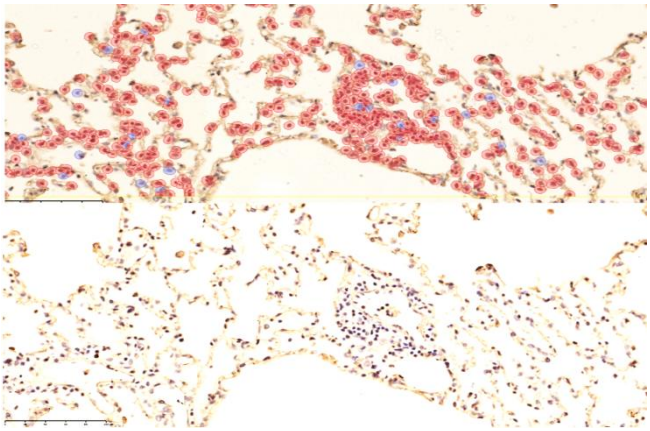

Figure 1. Lung tissue of a rabbit from the group exposed to Nurinol for 30 days without subsequent treatment. A low level of positive Bcl-2 expression is observed. The sample was scanned using QuPath software version 0.4.0.ink, which was used to determine the degree of expression. Bcl-2–positive cells are visualized as dark red staining. The staining was performed using DAB chromogen. Magnification: 10×10

Group 2 – 30-Day Nurinol Exposure with Alpha-Lipoic Acid

In stark contrast, lung tissue from Group 2 animals receiving ALA showed significantly enhanced Bcl-2 expression. In the representative sample analyzed, 312 total cells were detected, among which 142 cells were Bcl-2 positive, accounting for approximately 32.0% of the total population—more than double the positivity seen in Group 1.

Semi-quantitative scoring revealed:

9 animals (56.25%) showed score 3 (strong expression),
5 animals (31.25%) had score 2 (moderate expression),
and 2 animals (12.5%) displayed score 1 (weak expression).

None of the animals in this group were Bcl-2 negative (score 0).

The distribution of immunostaining was consistent and widespread, with dense DAB positivity (dark brown) prominently localized to the cytoplasm of bronchiolar epithelial cells and, to a lesser extent, alveolar lining cells. These findings strongly support the conclusion that ALA treatment preserved and restored the expression of Bcl-2, thereby enhancing the intrinsic cellular capacity to withstand oxidative and apoptotic stress.

The combined histological and immunohistochemical findings demonstrate that 30-day inhalation of Nurinol exerts a toxic effect on pulmonary cells, resulting in structural damage and suppression of protective molecular pathways. The significant upregulation of Bcl-2 in the ALA-treated group suggests that alpha-lipoic acid is capable of attenuating the cellular consequences of pesticide exposure, likely by stabilizing mitochondrial membranes, scavenging reactive oxygen species, and preventing the release of pro-apoptotic factors such as cytochrome c.

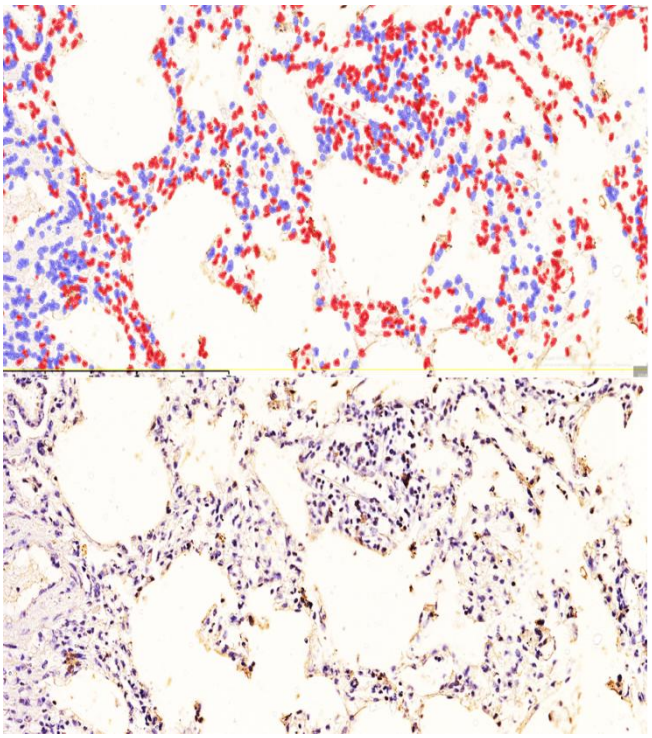

Figure 2. Lung tissue of a rabbit from the group that received Nurinol for 30 days followed by antioxidant treatment. A high level of positive Bcl-2 expression is observed. The sample was scanned using QuPath software version 0.4.0.ink, which was used to determine the expression level. Bcl-2–positive cells are stained dark red. Staining was performed using DAB chromogen. Magnification: 10×10

Taken together, these observations support the hypothesis that ALA confers a cytoprotective effect, maintaining epithelial integrity and enhancing the anti-apoptotic resilience of lung tissue under chemical stress. These results may have important implications for the development of antioxidant-based interventions in pesticide-induced respiratory toxicity.

4. Discussion

This study demonstrates that 30-day inhalation exposure to the pesticide Nurinol suppresses the expression of the anti-apoptotic protein Bcl-2 in rabbit lung tissue, leading to increased epithelial injury and signs of early fibrotic remodeling. In untreated animals, Bcl-2 levels were significantly reduced, suggesting that oxidative stress overwhelmed the intrinsic anti-apoptotic mechanisms.

This is consistent with known mechanisms of pesticide-induced apoptosis, where reactive oxygen species (ROS) cause mitochondrial damage, activate pro-apoptotic proteins like Bax, and trigger the release of cytochrome c and caspases —processes normally inhibited by Bcl-2 Youle & Strasser, 2008, Kroemer et al., 2007. Studies have shown that organophosphates such as chlorpyrifos reduce the Bcl-2/Bax ratio in lung and liver tissues, favoring apoptosis Abdollahi et al., 2004, El-Missiry et al., 2021.

In contrast, animals that received alpha-lipoic acid during the same exposure period showed significantly higher Bcl-2 expression and preserved lung architecture. ALA likely exerted a cytoprotective effect by: scavenging ROS and reducing oxidative burden [6], regenerating endogenous antioxidants like glutathione and vitamin C [9], stabilizing mitochondrial membranes, preventing cytochrome c release [10], enhancing Bcl-2 expression and reducing Bax-mediated apoptosis.

Our findings align with previous research showing that alpha-lipoic acid can restore Bcl-2 expression and protect against mitochondrial-driven apoptosis in various models of chemical toxicity, including arsenic-induced neurotoxicity and diabetic organ damage [11] [12].

Morphologically, lungs from alpha-lipoic acid -treated animals showed more viable bronchiolar epithelial cells, reduced inflammation, and higher Bcl-2 staining intensity. These results confirm that alpha-lipoic acid helps preserve lung structure and function by enhancing anti-apoptotic defenses at the mitochondrial level.

Although the study did not include an untreated control group, the clear contrast between treated and untreated groups supports the conclusion that alpha-lipoic acid provides effective protection against pesticide-induced lung injury by upregulating Bcl-2 and preserving epithelial integrity.

In conclusion, short-term exposure to Nurinol impairs anti-apoptotic protection in the lungs, as shown by suppressed Bcl-2 expression and structural damage. Alpha-lipoic acid co-treatment restores Bcl-2 expression, mitigates tissue injury, and may serve as a viable antioxidant intervention in acute pesticide exposure scenarios.

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