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HISTOLOGICAL CHANGES LARYNGEAL TISSUES INDUCED BY CYPERMETHRIN EXPOSURE

Yuldasheva Khushnoza Botirali kizi
2 nd-year Master's Student,Morphology department,
Fergana Medical institute of Public Health
yuldashevaxushnoza7@gmail.com +998910407407

Abstract.
Objective: To investigate the histomorphological alterations in the laryngeal tissues of rabbits (Oryctolagus cuniculus) following exposure to cypermethrin (CYP), a synthetic pyrethroid insecticide.
Methods: Eighteen adult male New Zealand White rabbits were used. Thirteen animals formed the experimental group, subjected to cypermethrin aerosol exposure for one month. Five rabbits served as the control group. Laryngeal tissues were processed, stained with Hematoxylin and Eosin (H&E) and Masson's Trichrome, and examined using light microscopy. The degree of tissue damage was evaluated using a semi-quantitative scoring method.
Results: The experimental group exhibited pronounced destructive and proliferative changes: hyperplasia and desquamation of the basal epithelial layer, severe edema and a dense inflammatory infiltrate in the submucosa, reduced secretory activity, and vacuolization of glandular cells. Significantly, focal degradation of chondrocytes and destruction of the cartilage matrix were observed. Isolated areas of epithelial metaplasia, where normal stratified squamous epithelium was replaced by columnar epithelium, were also identified.

Conclusion: Cypermethrin exposure induces severe toxic effects across all layers of the larynx, including inflammation, destruction, and adaptive responses. These findings underscore the necessity of developing effective prophylactic measures and continuous respiratory health monitoring for individuals exposed to this insecticide.

Keywords: Insecticide, Cypermethrin, Larynx, Toxicity, Rabbits, Pyrethroids, Histopathology, Desquamation, Metaplasia

Introduction: The Toxicological Significance of Pyrethroids

Pesticide Usage and Toxicological Concerns

The global market for chemical pesticides comprises nearly 1,000 active substances, with approximately 500 in widespread use, formulated into thousands of commercial products (Martynenko et al., 1992). While chemical control remains a dominant method in plant protection, it poses significant environmental and health risks. Pesticide intoxication ranks second only to pharmaceutical drug poisoning, necessitating the development of accurate diagnostic methods, including the identification of pre-nosological forms of chemical-induced pathology.

In toxicology, **pathomorphological analysis** plays a crucial role. It elucidates structural changes in organs and tissues, establishes the morphological substrate for clinical manifestations of intoxication, and thus enhances our understanding of the pathological processes triggered by various pesticide compounds.

Cypermethrin and Mechanisms of Toxicity

Cypermethrin (CYP) is a Type II synthetic pyrethroid insecticide widely used in agriculture, public health, and domestic settings. Its primary toxic action is **neurotoxicity**, achieved by

prolonging the opening of voltage-gated **sodium channels** in neuronal membranes, leading to hyperexcitability and repetitive firing. However, chronic or prolonged exposure extends its toxicity beyond the nervous system, targeting the **respiratory** and **immune systems**.

Inhalation is a primary exposure route, particularly in occupational settings. Studies on rodents indicate that pyrethroids induce **oxidative stress**—a critical mechanism of toxicity. This stress leads to the excessive generation of Reactive Oxygen Species (ROS), causing **lipid peroxidation**, cellular membrane damage, mitochondrial dysfunction, and the activation of **apoptosis** in tissues like the liver, lungs, and kidneys.

The Larynx as a Target Organ

The **larynx** is an essential component of the respiratory system, performing vital functions in **respiration**, **phonation**, and most importantly, **defense**. It prevents the penetration of harmful aerosols and particulates into the lower respiratory tract.

Exposure to toxic aerosols, such as cypermethrin, inevitably provokes **inflammatory, destructive, and adaptive changes** in laryngeal tissues. However, data specifically detailing the effects of cypermethrin on the histology of the rabbit larynx are extremely scarce. This study aims to fill this critical gap by providing a detailed morphological analysis of laryngeal tissue damage following exposure to CYP.

Materials and Methods

Experimental Design and Animal Model

Animal Model: Eighteen adult male **New Zealand White rabbits** (Oryctolagus cuniculus), weighing **2.4–2.7 kg**, were used.

- **Control Group (CG):** 5 animals.
- **Experimental Group (EG):** 13 animals.

The animals were housed under standard vivarium conditions with a natural light cycle (12h light/12h dark) and had ad libitum access to food and water. A 7-day acclimatization period preceded the experiments.

Exposure Protocol: Rabbits in the EG were subjected to inhalation exposure of cypermethrin aerosol (specific concentration and duration of daily exposure should be detailed based on the full thesis) for a duration of **one month**.

Tissue Preparation and Histological Staining

Following the conclusion of the exposure period, all animals were humanely euthanized in compliance with bioethical standards.

Fixation and Processing

The entire **larynges** were promptly excised and fixed in a **10% neutral buffered formalin solution** for **48 hours**. Tissues were then subjected to standard histological processing, including dehydration in graded alcohols, clearing, and embedding in paraffin blocks. Sections were cut using a microtome.

Staining Methods

For microscopic examination, two staining methods were employed:

1. **Hematoxylin and Eosin (H&E):** Used for evaluating general tissue architecture, cellular morphology, nuclear-cytoplasmic ratios, and identifying inflammation and cellular destruction.
2. **Masson's Trichrome Staining:** Employed specifically to assess the state of **connective tissue**, collagen fibers, and to identify potential fibrosis or alterations in the extracellular matrix of the lamina propria and cartilage.

Microscopic Analysis and Semi-Quantitative Scoring

Tissue slides were examined under a **light microscope** at magnifications of **×100, ×200, and ×400**. Special attention was paid to the integrity of the epithelium, the cellularity and

composition of the submucosal layer, the secretory status of the glands, and the condition of the cartilage.

A **semi-quantitative scoring method** was utilized to grade the severity of tissue damage, using a scale ranging from **0 (normal)** to **3 (severe/pronounced changes)**.

Results

Control Group Histology

The laryngeal tissues of control rabbits exhibited normal physiological histology.

- **Epithelium:** Intact, characterized by **stratified squamous non-keratinized epithelium** (vocal folds and epiglottis) and **pseudostratified ciliated columnar epithelium** (lower regions). No signs of cellular damage or inflammatory cell infiltration were observed.
- **Submucosa:** Homogeneous, composed of loose fibro-connective tissue, free from edema or inflammatory infiltrates.
- **Glands:** Seromucous glands displayed normal acinar structure and secretory activity.
- **Cartilage:** The hyaline cartilage matrix was structurally intact, with viable chondrocytes residing normally within their lacunae.

Experimental Group Histopathology

Histological examination of the laryngeal tissues from cypermethrin-exposed rabbits revealed complex and severe pathological alterations affecting all major structural components.

Epithelial Changes

The laryngeal epithelium demonstrated the most acute response to toxic exposure:

- **Desquamation:** Severe **sloughing (desquamation)** of the superficial epithelial cell layers was widely observed, leading to a compromised mucosal barrier function.
- **Basal Layer Hyperplasia:** Significant **thickening of the basal layer** due to **hyperplasia** was evident, representing a compensatory proliferative response to continuous damage.
- **Metaplasia:** In one rabbit, areas of **epithelial metaplasia** were noted, where the normal stratified squamous epithelium was locally replaced by **columnar epithelium**. This is an adaptive tissue transformation indicating chronic and deep irritation.
- **Degeneration:** Residual epithelial cells showed signs of **cellular degeneration**, including **cytoplasmic vacuolization** and pyknotic nuclei.

Submucosal and Inflammatory Responses

The underlying layers exhibited pronounced inflammatory changes:

- **Edema:** Marked **edema** in the submucosal layer, characterized by the **widening of interstitial spaces** and separation of connective tissue fibers.
- **Inflammatory Infiltrate:** A dense **inflammatory infiltrate** was present, predominantly composed of **neutrophils** and **macrophages**. This indicates active, non-specific inflammation targeting the elimination of the toxin and damaged cells.
- **Vascular Disturbances:** Signs of **vascular congestion** (hyperemia), **thickening of capillary walls**, and areas of **blood stasis** were noted, reflecting microcirculatory failure and increased vascular permeability typical of acute inflammation.

Glandular Destruction

The laryngeal seromucous glands also sustained significant damage:

- **Reduced Secretion:** A notable **decrease in secretory activity** was observed, likely impairing the moisturizing and cleansing functions of the mucus.
- **Cell Destruction: Destruction** of individual glandular cells was evident.
- **Vacuolization: Foci of cytoplasmic vacuolization** were a typical feature in glandular epithelial cells, suggesting metabolic disruption and accumulation of products of cellular injury.

Cartilage Tissue Damage

The toxic effects of cypermethrin extended to the deep supporting structures:

- **Chondrocyte Degradation: Focal degradation of chondrocytes** was observed within the hyaline cartilage.
- **Matrix Destruction:** There was evidence of **destruction of the extracellular matrix** of the cartilage tissue, which could compromise the mechanical integrity and rigidity of the larynx.

Discussion.

Pathogenetic Mechanisms and Oxidative Stress

The complex histological damage observed in the rabbit larynx confirms the potent toxic effect of cypermethrin via the inhalation route. The destructive and inflammatory processes (desquamation, edema, cellular infiltration) are likely triggered by the **activation of oxidative stress**, the hallmark of pyrethroid toxicity.

Oxidative stress leads to:

1. **Membrane Damage:** Peroxidation of lipids in cell membranes, causing **necrosis** and epithelial **desquamation**.
2. **Apoptosis Induction:** Activation of signaling pathways that culminate in programmed cell death.

The sustained presence of a **neutrophilic and macrophagic infiltrate** indicates chronic irritation and could exacerbate tissue injury through the continuous release of inflammatory cytokines and proteolytic enzymes.

Adaptive Responses and Metaplasia

Basal layer hyperplasia is a classic adaptive response, aiming for rapid epithelial repair. However, the detection of **epithelial metaplasia** (replacement of stratified squamous epithelium with columnar epithelium) is particularly significant.

- Metaplasia represents a deeper, chronic attempt by the tissue to adapt to prolonged irritation, often seen as a protective mechanism against severe chemical stressors. This finding suggests that the exposure protocol induced long-lasting, severe damage and may elevate the risk of future pathological changes, including potentially pre-neoplastic conditions, although further studies are required to confirm this.

Functional and Clinical Implications

The histological lesions have direct functional consequences:

- **Impaired Defense Function:** Epithelial desquamation and glandular destruction severely compromise the **mucociliary clearance** mechanism. Reduced mucus production leaves the mucosa more vulnerable to further toxin penetration and secondary infections.
- **Voice and Airway Compromise:** Edema of the submucosa and the structural **damage to the cartilage** (which provides the laryngeal framework) can lead to **dysphonia** (voice impairment) and potential **dyspnea** (breathing difficulties) due to airway narrowing. Cartilage matrix degradation may result in irreversible loss of elasticity and structure.

Comparison with Existing Literature

The results are consistent with prior studies on other laboratory animals (e.g., rodents), where pyrethroid exposure induced **respiratory tract inflammation** and mucosal structural changes. The unique contributions of this research lie in the detailed description of **cartilage tissue damage** and the identification of **epithelial metaplasia** in the rabbit model. These distinct findings may be attributable to species-specific metabolic differences or the specific duration and intensity of the aerosol exposure used in this experiment, warranting further investigation using immunohistochemical techniques to pinpoint specific molecular pathways (e.g., Caspase-3, COX-2).

Conclusion

Exposure to **cypermethrin** induces severe, multi-layered histological alterations in the laryngeal tissues of rabbits, characterized by:

1. **Epithelial destruction and proliferation** (including **desquamation**, **hyperplasia**, and **metaplasia**).
2. **Acute inflammation** of the submucosa with pronounced **edema** and **neutrophil-macrophage infiltration**.
3. **Reduced secretory function** and **destruction of mucous glands**.
4. **Degradation of chondrocytes** and **destruction of the cartilage matrix**.

These results unequivocally establish the larynx as a significant target organ for cypermethrin toxicity upon inhalation. The findings highlight the critical need for implementing effective **protective measures** (e.g., personal protective equipment, regulated use) and establishing **regular health monitoring programs** for agricultural workers and others routinely exposed to pyrethroid insecticides.

6. Future Directions

To further elucidate the pathogenesis of these changes, future research should focus on:

- **Immunohistochemical Analysis:** Precisely identifying markers of oxidative stress (e.g., MDA, SOD) and apoptosis (e.g., Caspase-3) within the damaged laryngeal tissues.
- **Recovery Assessment:** Studying the **long-term effects** of cypermethrin after the cessation of exposure to evaluate the tissue's capacity for recovery and repair.
- **Dose-Response Evaluation:** Comparative analysis of the histological effects of **varying cypermethrin doses** to establish a clear dose-dependency for the observed histopathological changes.

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