Research Article

The Journal of Anatomical Sciences
Email: journalofanatomicalsciences@gmail.com

J. Anat Sci 17(3) Sept.

Submitted: February 14th, 2026
Revised: March 24th, 2026
Accepted: June 30th, 2026

DOI: https://dx.doi.org/10.4314/jans.v17i3.4

Sex-Specific Neuroinflammation and Apoptotic Outcomes in the Cerebellum of Wistar Rats Exposed to λ-Cyhalothrin

Olanrewaju A. Danwahab [1,2*], Raji Maryam[1], Zainab Ibrahim[1], Joseph O. Oyeniyi [1], Mufutau O. Oladimeji [1], Ridwan Olanrewaju [1], Precious Omole [1], Priscilla Oloyede, Moyosore S. Ajao[1], Imam Aminu[1]

[1]Neuroscience Unit, Department of Anatomy, College of Health Sciences, University of Ilorin, Ilorin; [2]Department of Anatomy, College of Health Sciences, Kwara State University, Malete, Nigeria.

***Corresponding Author:** Email: danwahab.ambali@kwasu.edu.ng:

ABSTRACT

Sex-related biological differences influence how the nervous system responds to toxic exposures. These differences may determine the severity and pattern of neuronal injury produced by environmental neurotoxicants such as insecticides. λ-Cyhalothrin (LCT), a widely used type II pyrethroid, has been associated with neurotoxicity through mechanisms involving oxidative stress, neuronal damage, and neuroinflammation. This study investigated sex-specific differences in cerebellar toxicity following exposure to LCT in Wistar rats. Forty-eight adult rats (24 males and 24 females) were divided into control, low-dose (2 mg/kg), and high-dose (4 mg/kg) groups and treated orally for ten consecutive days. Neuromuscular performance was assessed using the hanging wire test. Cerebellar cytoarchitecture was evaluated using hematoxylin and eosin staining, while neuroinflammatory responses and glial integrity were assessed through immunohistochemical detection of tumor necrosis factor-alpha (TNF-α), glial fibrillary acidic protein (GFAP), and ionized calcium-binding adaptor molecule-1 (Iba1). Male rats showed no significant change in forelimb strength, whereas females demonstrated reduced latency to fall following low-dose exposure. Immunohistochemical analysis revealed increased activation of microglia and astrocytes in males, particularly at the lower dose, indicating an early neuroinflammatory response. In contrast, female rats exhibited limited glial activation but showed a dose-dependent increase in TNF-α expression within the cerebellum. These findings indicate that male rats display greater susceptibility to LCT-induced cerebellar neurotoxicity through glial activation, whereas females demonstrate a different inflammatory profile. The results highlight the importance of considering sex as a biological variable when assessing the neurotoxic effects of pesticides.

Keywords: λ-cyhalothrin, neurotoxicity, sex differences, cerebellum, neuroinflammation

INTRODUCTION

Pesticides are indispensable tools in modern agriculture and public health; however, their mechanisms of action often target biological pathways conserved across species, leading to unintended toxicity in non-target organisms, including humans[1]. This is particularly concerning for neurotoxic insecticides like organochlorines, organophosphates, and pyrethroids, which are designed to attack the nervous system of pests. The fundamental similarities between the insect and vertebrate nervous systems make cross-toxicity a significant risk [2]. Despite their regulated use, widespread human exposure to these compounds has intensified concerns about their potential adverse effects on brain health and cognitive function [3].

Emerging evidence highlights neuroimmunity and neuroepigenetics as key mediators in the development of sexually dimorphic brains. These processes contribute to enduring differences between males and females in neurochemical profiles, synaptic organization, neurogenesis, and regional brain volumes. Consequently, the multifaceted nature of sexual dimorphism presents a spectrum of distinct molecular targets for neurotoxicants, offering a biological rationale for the sex-specific effects of compounds like pyrethroids. The organizational and activational roles of steroid hormones, including estrogens, progesterone, and androgens, are fundamental in establishing and regulating these sex-based structural, functional, and behavioral differences throughout the lifespan [4].

As a type II pyrethroid, λ-cyhalothrin (LCT) exerts its neurotoxic effects through multiple mechanisms.

While its primary action is on voltage-gated sodium channels (VGSCs), it also modulates chloride, calcium, and potassium channels. Furthermore, LCT can disrupt the activity of glutamate and acetylcholine receptors and inhibit adenosine triphosphatases, ultimately leading to oxidative stress and DNA damage within neurons[5]. It also alters the function of key neurotransmitters such as gamma-aminobutyric acid (GABA) and dopamine. In humans, acute high-dose exposure can manifest as excessive salivation, fatigue, coughing, and abdominal pain[6]. Due to their lipophilic nature, pyrethroids like LCT readily cross the blood-brain barrier, achieving concentrations sufficient to induce neurotoxicity by prolonging the opening of VGSCs[7].

Microglia, the resident immune cells of the central nervous system (CNS), are central to neuroinflammatory processes associated with various neurodegenerative conditions. Repeated or high-dose pyrethroid exposure has been directly linked to glial cell activation and subsequent neuroinflammation in vivo [8,9]. The presence of VGSCs on microglial membranes provides a direct pathway for pyrethroid action. Activation of these channels can lead to an excessive influx of sodium ions, triggering the release of major pro-inflammatory cytokines, notably tumor necrosis factor-alpha (TNF-α) [9]. This cascade can disrupt glial homeostasis, leading to altered expression of markers like GFAP (for astrocytes) and Iba1 (for microglia), and amplifying neuroinflammation [8]. Additionally, neurotoxic insults, such as insecticide exposure, are known to upregulate pro-apoptotic proteins like BAX, which can initiate programmed cell death. Within the cerebellum, Purkinje neurons in the ganglionic layer appear particularly vulnerable to this type of insult [10; 11; 12; 13].

Therefore, the rationale for this study was to investigate whether exposure to λ-cyhalothrin produces sex-dependent neurotoxic and neuroinflammatory responses in the cerebellum. The aim of this study was to evaluate sex-specific changes in neuromuscular performance, cerebellar cytoarchitecture, and glial activation following oral exposure to λ-cyhalothrin in Wistar rats.

MATERIALS AND METHODS

Ethical approval

All experimental procedures received prior approval from the University of Ilorin Ethical Review Committee (UERC) (approval number: UIL/UERC/11/46KA072). The study was conducted in strict adherence to the guidelines and recommendations of the College of Health Sciences Ethical Review Committee and followed the principles outlined in the Institutional Animal Care and Use Committee (IACUC) guide.

Animals and experimental design

Forty-eight (48) adult Wistar rats, comprising 24 males and 24 females with an average weight of 180 ± 20 g, were obtained from the University of Ilorin Biological Garden. The animals were housed in standard cages within the animal holding facility of the Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin. They were maintained under a 12-hour light/dark cycle at room temperature and provided with standard laboratory chow and water ad libitum. All animals were acclimatized for seven days prior to the commencement of the experiment. Animal care and handling were performed in accordance with established guidelines for the ethical use of laboratory animals.

Treatment plan

Following acclimatization, rats of each sex were randomly assigned to one of three experimental groups (n=8 per group per sex):

Control Group**:** Received a daily oral dose of corn oil (1 ml/kg body weight), which served as the vehicle.

Low-Dose LCT Group**:** Received a daily oral dose of λ-cyhalothrin (2 mg/kg body weight).

High-Dose LCT Group**:** Received a daily oral dose of λ-cyhalothrin (4 mg/kg body weight).

All treatments were administered via oral gavage for a period of ten (10) consecutive days.

Behavioral assessment: Hanging wire test

To evaluate the effect of LCT on neuromuscular function, rats were subjected to the hanging wire test on days 10 and 11 of the treatment period. This test assesses forelimb muscular strength and motor deficits in rodent models. Each animal was placed on an elevated wire cage top and allowed to grip the wire. The latency to fall was recorded, with a longer latency indicating greater muscular strength.

Animal euthanasia and sample collection

Twenty-four hours after the final treatment, rats were euthanized with an intramuscular injection of ketamine (20 mg/kg). Upon confirmation of complete immobility and loss of response to tactile stimuli, a thoracotomy was performed to expose the heart. Systemic circulation was cleared by making a small incision in the right atrium and transcardially perfusing 20 ml of normal saline through the left ventricle. This was immediately followed by perfusion with 50 ml of 10% buffered formalin for whole-body fixation.

Brain harvest and tissue processing

Following perfusion, the skull was opened by carefully removing the dorsal portion to expose the brain. The dura mater, optic nerves, and other cranial nerve attachments were severed to release the brain from the cranial base. The harvested brains were then post-fixed in fresh 10% buffered formalin. Cerebellar

tissues were subsequently dissected, processed through a graded series of alcohols and xylene, and embedded in paraffin wax.

Serial sections of the cerebellum, cut at a thickness of 5 μm, were obtained for the following staining procedures:

Hematoxylin and eosin (H&E) staining: Sections were deparaffinized, rehydrated, and stained with hematoxylin to visualize cell nuclei and eosin to stain the cytoplasm and extracellular matrix. This provided an overview of the general cerebellar cytoarchitecture.

Immunohistochemistry (IHC): To assess glial activation and neuroinflammation, sections were stained for Iba1 (microglial marker), GFAP (astrocytic marker), and TNF-α (pro-inflammatory cytokine). Following deparaffinization, rehydration, and heat-mediated antigen retrieval in citrate buffer (pH 6.0), endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide. Non-specific protein binding was blocked with 2.5% normal horse serum. Sections were then incubated overnight at 4°C with primary antibodies: goat polyclonal anti-Iba1 (1:250, Abcam, ab5076), mouse monoclonal anti-GFAP-HRP conjugated (1:150, Santa Cruz, sc-33673), and rabbit polyclonal anti-TNF-α (1:200, Novus Biologicals, NB120-11427). After washing, appropriate ImmPRESS™ HRP Polymer Reagents (Vector Labs) were applied. Immunoreactivity was visualized using a DAB Peroxidase (HRP) Substrate Kit (Vector Labs), and sections were counterstained with hematoxylin.

Microscopy, photomicrography, and image analysis

Stained sections were examined and photographed using an AmScope compound microscope equipped with a 5.0 MP digital camera (iSCOPE Corp., USA). Images were captured at 400x magnification. Quantitative analysis of stained sections was performed using ImageJ software (NIH, USA). For cell counting, a grid was overlaid on the images, and specifically stained cells (e.g., Iba1-positive microglia, GFAP-positive astrocytes) were manually counted. For staining intensity, images were converted to RGB stacks, and the mean gray value of the thresholded area corresponding to the immunoreactive signal (e.g., TNF-α) was measured.

Statistical analysis

All quantitative data were analyzed using GraphPad Prism (Version 8.4.2). A two-way analysis of variance (ANOVA) was performed to assess the effects of sex and treatment, followed by Tukey's multiple comparisons post-hoc test. Statistical significance was set at $p \geq 0.05$. Results are presented as scatter plots and bar charts showing the mean and standard deviation.

RESULTS

Forelimb muscular strength

The hanging wire test revealed a sex-dependent effect of LCT on muscular strength (Figure 1). In male rats, low-dose LCT exposure was associated with a non-significant trend toward increased time on the wire compared to controls ($p = 0.461$), suggesting a possible enhancement of muscle function. This effect diminished at the high dose, with hanging times returning to near-control levels ($p > 0.999$). Conversely, female rats exhibited a reduction in muscular strength following low-dose LCT exposure, indicated by a shorter latency to fall ($p = 0.149$). However, at the high dose, their performance improved, showing a longer hanging time compared to the low-dose group ($p = 0.388$), indicating a non-linear, non-dose-dependent response in females (Figure 1).

Neuroinflammatory responses and glial integrity

Activated microglia cells in the cerebellar cortices of λ-cyhalothrin exposed rats

Microglial activation in the cerebellum following λ-cyhalothrin exposure demonstrated distinct sex-specific patterns of microglial activation (Figure 3). In control animals of both sexes, microglia displayed a ramified, resting morphology. Following low-dose LCT exposure, male rats showed a rapid and significant increase in the number of activated (amoeboid) microglia, indicating a neuroinflammatory response. Interestingly, Iba1 expression in males was significantly lower in the high-dose group compared to the low-dose group ($p = 0.006$), although it remained elevated relative to controls ($p < 0.001$). This suggests a potential compensatory or regulatory mechanism at higher doses.

In contrast, female rats showed no significant change in Iba1 expression at the low dose compared to controls ($p > 0.99$). A trend towards increased microglial activation was only observed at the high dose, though this did not reach statistical significance ($p = 0.11$ vs. control; $p = 0.46$ vs. low dose). This indicates a delayed and blunted microglial response in females compared to males (Figure 3).

Reactive astrocytes in the cerebellar cortices of λ-cyhalothrin exposed rats

Analysis of GFAP expression revealed a dose-dependent increase in reactive astrocytes in both sexes, but with different onset thresholds (Figure 4). In male rats, a significant increase in GFAP-positive astrocytes was evident even at the low dose ($p = 0.01$ vs. control), with a further significant elevation at the high dose ($p < 0.001$ vs. low dose), demonstrating a clear dose-response relationship. In female rats, no significant reactive astrogliosis was observed at the low dose ($p > 0.99$ vs. control). A significant increase in GFAP expression was only apparent following high-dose LCT exposure ($p = 0.002$ vs. control; $p = 0.004$ vs. low dose). This reinforces the finding that male rats mount a more rapid glial response to LCT neurotoxicity (Figure 4).

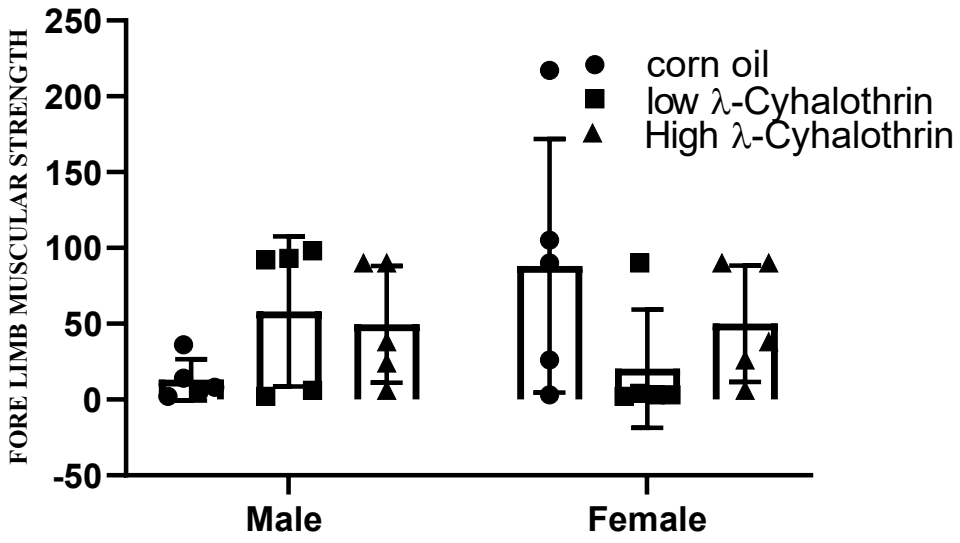

Figure 1: Graph showing the muscular strength of forelimb behavior of rats using hanging wire after exposure to corn oil, low and high λ-cyhalothrin.

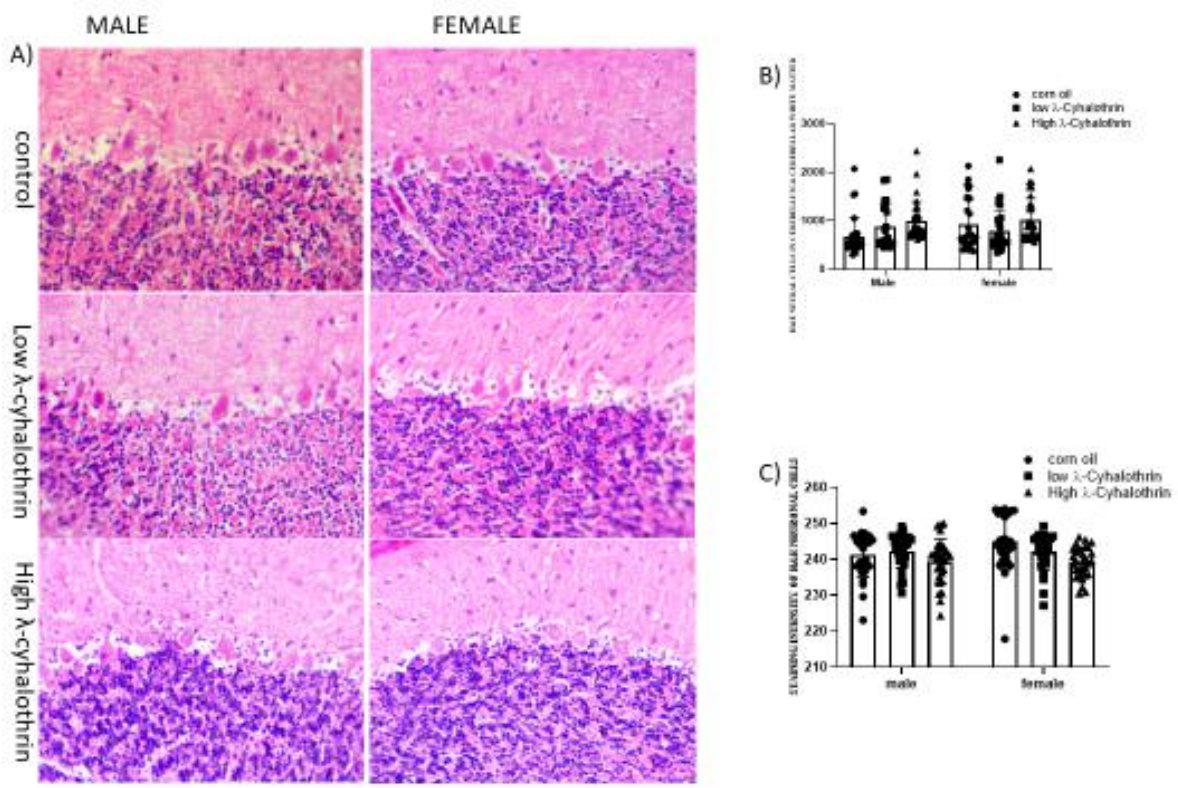

Figure 2: Representative photomicrographs of H&E-stained cells (A), Estimation of neuronal cells (B), and the intensity of the stain (C), in the cerebellar cortices of rats exposed to 2 mg/kg (low) and 4 mg/kg (high) λ-cyhalothrin. Anti-IBA1 antibody, X400, 25 μm.

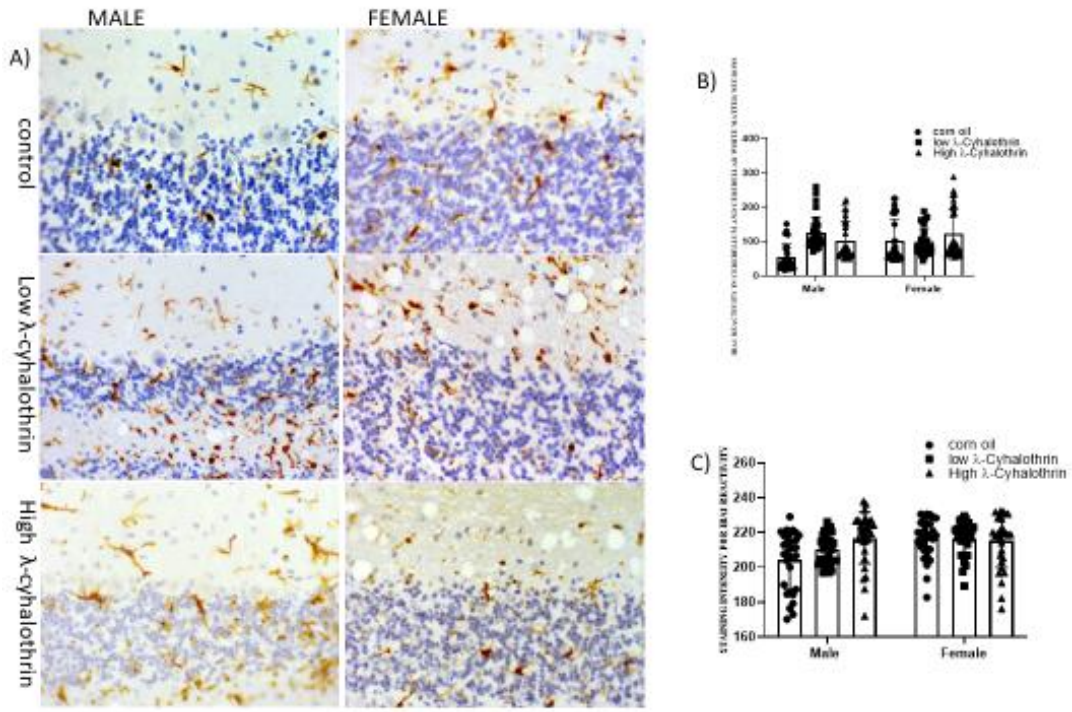

Figure 3: Representative photomicrographs of IBA1-expressing microglia (A), Estimation of activated microglia (B), and the intensity of IBA1 expression (C), in the cerebellar cortices of rats exposed to 2 mg/kg (low) and 4 mg/kg (high) λ-cyhalothrin. Anti-IBA1 antibody, X400, 25 µm

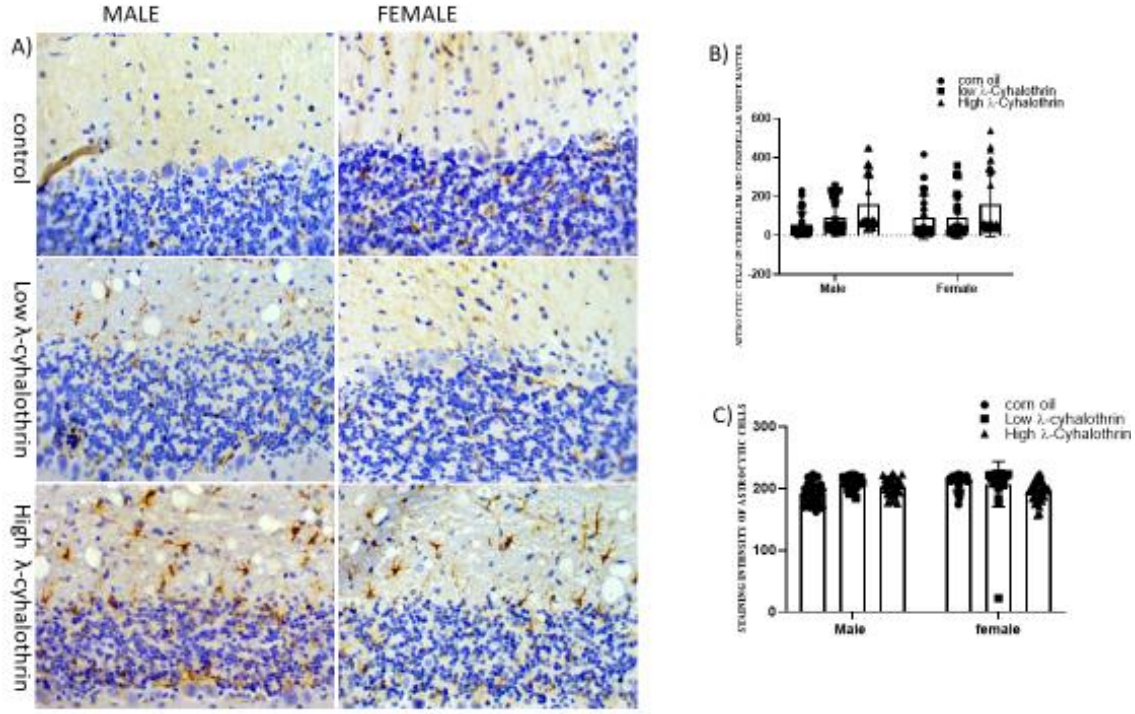

Figure 4: Representative photomicrographs of GFAP-expressing astrocytes (A), Estimation of reactive astrocytes (B), and the intensity of the GFAP expression (C), in the cerebellar cortices of rats exposed to 2 mg/kg (low) and 4 mg/kg (high) λ-cyhalothrin. Anti-IBA1 antibody, X400, 25 µm.

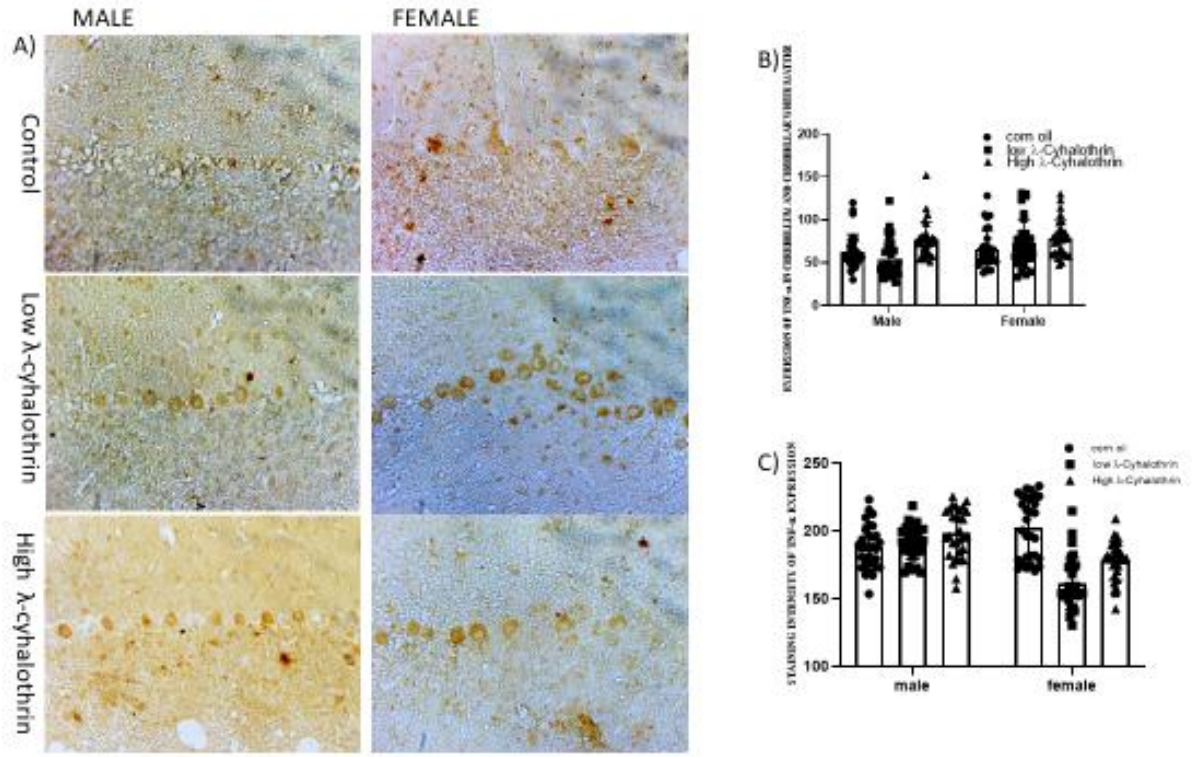

Figure 5: Representative photomicrographs of IBA1-expressing microglia (A), Estimation of activated microglia (B), and the intensity of IBA1 expression (C), in the cerebellar cortices of rats exposed to 2 mg/kg (low) and 4 mg/kg (high) λ-cyhalothrin. Anti-IBA1 antibody, X400, 25 µm

Tumor necrosis factor-α expression in the cerebellar cortices of λ-cyhalothrin exposed rats

The expression of the pro-inflammatory cytokine TNF-α also showed a sex-dependent pattern (Figure 5). In male rats, TNF-α expression in the low-dose group was slightly, but not significantly, elevated compared to controls ($p = 0.176$). A significant and sharp increase was observed in the high-dose group compared to the low-dose group ($p < 0.001$), indicating a strong pro-inflammatory response at the highest exposure level. In female rats, a steady, dose-dependent increase in TNF-α expression was observed across the control, low-dose, and high-dose groups. However, these increases were not statistically significant ($p = 0.249$ for low vs. control; $p = 0.568$ for high vs. low), suggesting a more gradual, albeit less intense, inflammatory response compared to males (Figure 5).

DISCUSSION

The widespread use of pyrethroid insecticides like λ-cyhalothrin (LCT) for agricultural and domestic pest control necessitates a comprehensive understanding of their potential neurotoxicity, particularly given the structural and functional similarities between the insect and mammalian nervous systems [14; 15]. This study provides critical evidence that LCT-induced neurotoxicity in the cerebellum is profoundly influenced by sex, with male rats displaying a more rapid and pronounced response at the glial level, while females exhibit a greater susceptibility in terms of motor function at lower doses.

Our behavioral data from the hanging wire test revealed a complex, sex-dependent effect on motor function. The non-significant increase in forelimb strength in low-dose males aligns with the established concept of hormesis or the excitatory potential of low-dose pyrethroid exposure on sodium channel function, which can transiently enhance muscle contraction [16]. However, the decline at higher doses in males and the overall reduced performance in females are consistent with the known toxic effects of high-dose pyrethroid exposure, which can lead to muscle weakness through prolonged sodium channel opening and subsequent neuronal dysfunction[17]. The pronounced deficit in female rats at the low dose, which improved at the high dose, suggests a non-monotonic response that may be modulated by hormonal factors, such as the neuroprotective effects of estrogen or the differential modulation of pyrethroid toxicity by androgens [18].

The histopathological and immunohistochemical findings provide mechanistic insight into the behavioral differences. The dose-dependent increase in cerebellar neuronal cell density observed with H&E staining in both sexes, particularly at high doses, may initially seem paradoxical. However, this could reflect cellular shrinkage, clustering due to edema, or an increase in non-neuronal cells like glia, rather than true neurogenesis, as a response to injury. This highlights the importance of using specific cellular markers, as we did with Iba1 and GFAP.

Our data strongly indicate that the male cerebellum is more sensitive to LCT-induced glial activation. The rapid and significant increase in activated microglia (Iba1+) and reactive astrocytes (GFAP+) in males at the low dose demonstrates a swift neuroinflammatory response. The decrease in microglial activation in males at the high dose, while astrogliosis continued to rise, is intriguing. It may suggest a phenotypic shift in microglia from a pro-inflammatory (M1) to an anti-inflammatory/repair (M2) state, or a depletion of the microglial response, while astrocytes continue their role in attempting to wall off the injury [19; 20]. The delayed glial response in females, with significant changes only appearing at the high dose, points towards a protective mechanism that dampens the initial response to neurotoxic insult, potentially mediated by sex hormones like estrogen, which is known for its immunomodulatory and neuroprotective properties [21].

The TNF-α expression patterns further support this sexually dimorphic inflammatory profile. Males exhibited a sharp, significant increase in this potent pro-inflammatory cytokine only at the high dose, suggesting that the early glial activation at low doses may not immediately translate into a full-blown cytokine storm. This contrasts with the female response, which, although more muted and non-significant, showed a steady, dose-dependent increase. The elevated TNF-α in both sexes at higher doses confirms the role of LCT in triggering oxidative stress and inflammatory pathways [22; 14; 23].

This study demonstrates that male rats are more vulnerable to LCT-induced cerebellar glial disruption and neuroinflammation, while females show greater sensitivity in terms of behavioral motor deficits at lower exposure levels. This dichotomy underscores the complex interplay between toxicant mechanism, neural substrate, and the modulatory role of sex. It reinforces the critical necessity of incorporating sex as a fundamental biological variable in neurotoxicological research to ensure the development of precise and effective risk assessment and therapeutic strategies.

CONCLUSION

Exposure to λ-cyhalothrin elicits sex-specific neurotoxic effects in the rat cerebellum. These findings highlight the importance of considering sex differences in understanding the mechanisms of pyrethroid toxicity and in developing targeted interventions.

REFERENCES

1. Richardson JR, Fitsanakis V, Westerink RHS, Kanthasamy AG. Neurotoxicity of pesticides. *Acta Neuropathol.* 2019;138(3):343-362.

2. Abreu-Villaça Y, Levin ED. Developmental neurotoxicity of succeeding generations of insecticides. Environ Int. 2017;99:55-77.
3. Elser BA, Hing B, Stevens HE. A narrative review of converging evidence addressing developmental toxicity of pyrethroid insecticides. Crit Rev Toxicol. 2022;52(5):371-388.
4. McEwen BS. Steroid hormones: effect on brain development and function. Horm Res. 1992;37 Suppl 3:1-10.
5. Ahamad A, Kumar J. Pyrethroid pesticides: an overview on classification, toxicological assessment and monitoring. J Hazard Mater Adv. 2023;10:100284.
6. Pitzer EM, Sugimoto C, Gudelsky GA, Huff Adams CL, Williams MT, Vorhees CV. Deltamethrin exposure daily from postnatal day 3-20 in Sprague-Dawley rats causes long-term cognitive and behavioral deficits. Toxicol Sci. 2019;169(2):511-523.
7. Hao F, Bu Y, Huang S, Li W, Feng H, Wang Y. Effects of pyrethroids on the cerebellum and related mechanisms: a narrative review. Crit Rev Toxicol. 2023;53(4):229–43.
8. Gargouri B, Yousif NM, Bouchard M, Fetoui H, Fiebich BL. Inflammatory and cytotoxic effects of bifenthrin in primary microglia and organotypic hippocampal slice cultures. J Neuroinflammation. 2018;15:19.
9. Hossain MM, Liu J, Richardson JR. Pyrethroid insecticides directly activate microglia through interaction with voltage-gated sodium channels. Toxicol Sci. 2017;155(1):112-123.
10. Baltazar MT, Dinis-Oliveira RJ, de Lourdes Bastos M, Tsatsakis AM, Duarte JA, Carvalho F. Pesticide exposure as an etiological factor of Parkinson's disease and other neurodegenerative diseases: a mechanistic approach. Toxicol Lett. 2014;230(2):85-103.
11. Ueda RMR, De Souza VM, Magalhães LR, Chagas PHN, Veras ASC, Teixeira GR, *et al*. Neurotoxicity associated with chronic exposure to dichlorophenoxyacetic acid (2,4-D) – a simulation of environmental exposure in adult rats. J Environ Sci Health Part B. 2021;56(8):695–705.
12. Zare Z, Zarbakhsh S, Mashhadban S, Moradgholi A, Mohammadi M. Apoptosis is involved in paraoxon-induced histological changes in rat cerebellum. Drug Chem Toxicol. 2022;45(6):2554–60.
13. Erekat NS. Programmed cell death in cerebellar Purkinje neurons. J Integr Neurosci. 2022;21(1):30. doi: 10.31083/j.jin2101030.
14. Nieradko-Iwanicka B, Konopelko M. Effect of lambda-cyhalothrin on locomotor activity, memory, selected biochemical parameters, tumor necrosis factor α, and interleukin 1β in a mouse model. Int J Environ Res Public Health. 2020;17(24):9240.
15. Ravula AR, Yenugu S. Pyrethroid-based pesticides: chemical and biological aspects. Crit Rev Toxicol. 2021;51(2):117-140.
16. Soderlund DM, Clark JM, Sheets LP, Mullin LS, Piccirillo VJ, Sargent D, *et al.* Mechanisms of pyrethroid neurotoxicity: implications for cumulative risk assessment. Toxicology. 2002;171(1):3-59.
17. Shafer TJ, Meyer DA, Crofton KM. Developmental neurotoxicity of pyrethroid insecticides: critical review and future research needs. Environ Health Perspect. 2005;113(2):123-136.
18. Wolansky MJ, Gennings C, Crofton KM. Relative potencies for acute effects of pyrethroids on motor function in rats. Toxicol Sci. 2006;89(1):271-277.
19. Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, *et al.* Neurotoxic reactive astrocytes are induced by activated microglia. Nature. 2017;541(7638):481-487.
20. Henry RJ, Ritzel RM, Barrett JP, Doran SJ, Jiao Y, Leach JB, *et al*. Microglial Depletion with CSF1R Inhibitor During Chronic Phase of Experimental Traumatic Brain Injury Reduces Neurodegeneration and Neurological Deficits. J Neurosci. 2020;(14):2960–74.
21. Thakkar R, Wang R, Wang J, Vadlamudi RK, Brann DW. 17 β -Estradiol Regulates Microglia Activation and Polarization in the Hippocampus Following Global Cerebral Ischemia. Oxid Med Cell Longev. 2018;(1):4248526.
22. Afolabi OK, Aderibigbe FA, Folarin DT, Arinola A, Wusu AD. Oxidative stress and inflammation following sub-lethal oral exposure of cypermethrin in rats: mitigating potential of epicatechin. Heliyon. 2019;9;5(8):e02274.
23. You K, Gu H, Yuan Z, Xu X. Tumor necrosis factor alpha signaling and organogenesis. Front Cell Dev Biol. 2021;9:727075.